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JOURNAL OF DAIRY SCIENCE

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COLOR DEVELOPMENT IN LACTOSE SOLUTIONS DURING HEATING WITH SPECIAL REFERENCE TO THE COLOR OF EVAPORATED MILK

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In the manufacture of those dairy products which are processed at high temperatures or which contain a large quantity of reducing sugar, an undesirable brown color develops. This color is especially noticeable in the manufacture of milk sugar, evaporated milk and sweetened condensed milk.

It is a matter of general experience in lactose manufacture that during the heating of the neutralized whey which contains considerable quantities of protein, a dark brown color develops which is greatly intensified as the reaction of the solution is made more alkaline.

In the case of evaporated milk, which contains approximately 10 per cent lactose and 6.7 per cent protein and is sterilized at 115° C. for fifteen minutes, a characteristic brown color develops during heating. Since the composition and processing of this product must be controlled within narrow limits, the possibility of avoiding a darkening in color appears slight. The brown color of evaporated milk is held by the casein after coagulation and cannot be washed from this protein nor removed from it by dissolving the coagulated and washed casein and re-precipitating it in acid solution.

Sweetened condensed milk, not being subject to extremely high processing temperatures, does not suffer excessive darkening in color during manufacture, but undergoes a marked color change in storage, especially at high temperatures (5).

There are many references in the literature dealing with the browning of sugar solutions during heating. If a pure sugar is heated dry or in an alkaline medium, caramel is formed, the reaction apparently being one of progressive dehydration and polymerization. If a reducing sugar is heated in alkaline or slightly acid solution in the presence of amino acids, a brown color develops, its intensity being chiefly dependent upon temperature, reaction of the medium and the amino acid and sugar concentration.

A reaction occurs at ordinary temperatures between reducing sugars and

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amino acids. The optical rotation of glucose shows a decrease upon the addition of glycine to the sugar solution (14).

The product formed when glucose is heated with an amino acid is complex in nature and has been shown to contain carbon, hydrogen, oxygen and nitrogen (10), (1).

There is evidence, especially in the case of cane sugar, that catalysts play an important rôle in the polymerization of sugars during heating. Metals, especially iron, have been found to catalyze the coloring of alkaline sucrose solutions at high temperatures, (3), (9), (11), (12), (13).

Caramel was prepared by Beal and Bowey (2) from glucose, using ammonium chloride or ammonium sulfate with hydrochloric acid. These salts catalyzed the reaction and did not seem to enter into the composition of the caramel.

Orla-Jensen and Plattner (7) first showed that the color which develops in heated milk could be attributed to the presence of both lactose and casein. Wright (17) considered the color to be due to a caramelization of the lactose which was catalyzed by calcium caseinate and that the casein in adsorbing the pigment remained unchanged.

Ramsey, Tracy and Ruehe (8) have reported some interesting experiments upon color development in skim milk subjected to various treatments. They consider the color to be due to a sugar-amino acid condensation, the speed of the reaction depending upon temperature, hydrogen-ion concentration, and the kind of sugar used.

Color changes caused by certain modifications in the manufacturing process of evaporated milk have been studied by Webb and Holm (15). They found that an increase in heat, whether encountered during forewarming, sterilization or storage, produced an increase in color, the change being of a catalytic nature.

EXPERIMENTAL

The work included in this report covers a series of experiments carried out on lactose solutions held during heat treatment at known pH values by means of suitable buffers. The color of the heated solutions was measured by comparison with color standards of known values.

Lactose solutions of the required concentration were made up from special lactose of a high degree of purity. Buffer solutions and solutions of amino acids and ammonium salts were prepared to give the desired molal concentration when mixed with a given quantity of lactose solution. All figures expressing concentration of lactose, buffers or salts in the different solutions are given as the concentration of the specified substance in the particular solution under consideration. The concentration of solutions from which the final mixtures were made have been omitted.

A total of 7 cc. of each mixture to be heated was measured into a pyrex

test tube, the upper part of which had previously been drawn out to facilitate sealing the tube. The tubes were sealed and heated in a glycerine bath held at 120° C. for different lengths of time. After rapid cooling, the color of the lactose solutions was measured by comparison with sealed tubes of standardized colors in a constant north light, using a special test tube rack with a white background. These comparisons were made by eye and it was found possible to check the measurements to $\frac{1}{3}$ to $\frac{1}{2}$ of the difference between any two standards. Therefore differences between standards are expressed as $\frac{1}{3}$, $\frac{1}{2}$ or $\frac{2}{3}$ as the case might be.

TABLE 1

The composition of the color standards used for measuring the color of heated lactose solutions

COLOR STANDARD NUMBER	COMPOSITION OF COLOR STANDARD EACH MADE UP TO 7 CC. WITH DISTILLED WATER
1	0.3 cc. of CPR–PR solution[1]
2	0.6 " " " " "
3	1.0 " " " " "
4	1.5 " " " " "
5	2.0 " " " " "
6	3.0 " " " " "
7	0.2 cc. Thymol Blue (0.04%) solution
8	0.3 " " " " "
9	0.45 " " " " "
10	0.7 " " " " "
11	1.1 " " " " "
12	1.5 " " " " "
13	2.0 " " " " "
14	7.0 cc. Molal Fe Cl_3
15	7.0 " 1½ " "
16	7.0 " 2 " "

[1] Solution consisted in 3.8 cc. chlor phenol red concentration 0.04%, 0.6 cc. of phenol red, concentration 0.04%, and 10 cc. of HCl made up to 100 cc. with distilled water.

The colors developed in the lactose solutions during heating and storage were compared with color standards prepared according to table 1. These colors ranged from an almost water-clear solution (#1) to the dark reddish brown of 2 molal Fe Cl_3 solution (#16). Reproduction of these colors is possible by following table 1. If difficulty is encountered with the thymol blue standards a very slight shift in the pH of the distilled water solutions will place them in their proper positions. The fact should be noted that different samples of Fe Cl_3 may give slightly different shades of brown, depending upon the extent to which Fe $(OH)_3$ has formed in the sample used.

To be certain that the differences between standards represented equal differences in color it seemed desirable to check the standards more accurately than it was possible to do by eye. Therefore measurements expressed

in numerical terms according to the Munsell system (6) (15) were secured. The measurements were made upon a duplicate set of standards, the originals having already been sealed in test tubes which were not of appropriate size to measure in the colorimeter. Measurements were made on one-inch diameter areas of liquid placed in culture flasks of 1¼ inches diameter backed by N 9.4 paper. To reduce the chroma in matching samples and color discs it was necessary to place a narrow strip of white across the sample which would make the corrected chroma approximately 10 per cent stronger than the values indicate.

The data obtained are shown in Fig. 1. It will be noted that, except

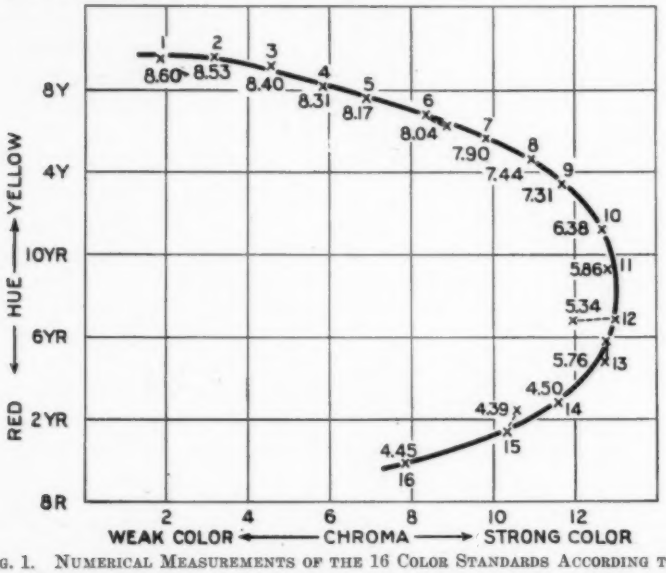

FIG. 1. NUMERICAL MEASUREMENTS OF THE 16 COLOR STANDARDS ACCORDING TO THE MUNSELL SYSTEM. THE FIGURES WITHIN THE CURVE REFER TO THE THIRD COLOR ATTRIBUTE, BRILLIANCE, AND THE GREATER VALUES REFER TO THE LIGHTER COLORS.

for small variations, the spacing of the standards along the curve is quite uniform, indicating the color difference between each to be approximately the same. The positions of standards 6, 12, 13, and 15 have been shifted to the curve from their measured positions because comparison by eye of the original sealed standards to the measured duplicates showed these positions to be more nearly those occupied by the sealed standards. In the present work the sixteen standards have been considered as being equally spaced over the range of color which they include.

The hydrogen-ion concentration of a lactose solution is of major importance in controlling color development during heating. This is especially true where the reaction is above pH 6.0; and when pH 7.0 is reached,

it requires very little heating to produce a deep caramel color. It was therefore deemed advisable to determine the pH of the solutions both before and after heating. These figures, determined potentiometrically by means of a quinhydrone electrode, are given in the accompanying tables and charts.

The effect of differences in pH can be noted in several of the following tables. The data given in table 2 show clearly the marked darkening in the color of 4 per cent heated lactose solutions as the pH is increased.

To counteract the acidity which develops in lactose solutions during heating and eliminate a variable in the experimental work, an efficient buffer is necessary. Several buffers were tried, the color and pH of each solution being measured after completion of the heating period.

TABLE 2

The relationship in color development of 4% lactose solutions heated to 120° C. for 45 minutes, when buffered by sodium phosphate and by sodium maleate, with and without the addition of ammonium chloride

BUFFER	No NH_4Cl			NH_4Cl CONCENTRATION = M/13		
	REACTION		COLOR	REACTION		COLOR
	Before heating	After heating		Before heating	After heating	
	pH	*pH*		*pH*	*pH*	
Sodium Phosphate buffer M/4.5	5.67	5.66	3–⅓	5.63	5.55	9–⅓
	5.90	5.84	4–⅔	5.87	5.76	12
	6.04	5.97	5–⅔	6.01	5.91	13
	6.27	6.19	7	6.24	6.12	14
	6.47	6.32	8–⅓	6.45	6.27	15
	6.66	6.45	9	6.63	6.41	16
	6.83	6.55	10	6.80	6.55	(17)
Sodium Maleate buffer M/1.5	5.47	5.47	1–⅓	5.42	5.40	7
	5.93	5.92	4–⅓	5.90	5.90	10–⅓
	6.23	6.22	6	6.20	6.15	13
	6.47	6.36	8	6.43	6.37	13–⅔
	6.54	6.38	8	6.51	6.40	14
	6.71	6.44	9	6.71	6.46	14–⅓

Some of the data obtained are plotted in Fig. 2. The figures on the ordinate are the product of the time of sterilization in minutes and the color intensity as expressed by the number of the color standard which the sample matched. The figures for evaporated milk are taken from the data of Webb and Holm (15) where different color standards were used.

Citrate and acetate were not satisfactory but phosphate and maleate buffers were very effective in maintaining a reaction comparable to that encountered in milk. Curves 3 and 4, representing M/2 maleate and M/2 phosphate solutions in 5 per cent lactose show strong buffering at the reaction of evaporated milk. If the lactose concentration is increased to 10 per cent as in curve 6, even molal phosphate is not sufficient to hold the

reaction constant. Much of the work reported below was carried out using M/2 phosphate and M/2 maleate solutions to buffer 5 per cent lactose.

The phosphate buffers were made up to the required pH by using appropriate proportions of $Na\,H_2PO_4$ and Na_2HPO_4. Maleate buffers were made up by adding concentrated NaOH to solutions of maleic acid of the specified concentration. Sufficient alkali was added to the maleic acid to give a solution of the desired pH. For maleic acid $pK_1 = 1.93$ and $pK_2 = 6.58$, (4), the buffering of the second hydrogen being the one of interest in this work.

There has been considerable uncertainty in dealing with the changes occurring in milk during heating as to whether the phosphates present in normal milk act in a catalytic capacity. Whittier and Benton (16) in their work on the formation of acid in milk by heating concluded in part, "Experiments on lactose solutions containing radicals similar in their buffer action to those in milk, but chemically different, would settle the question

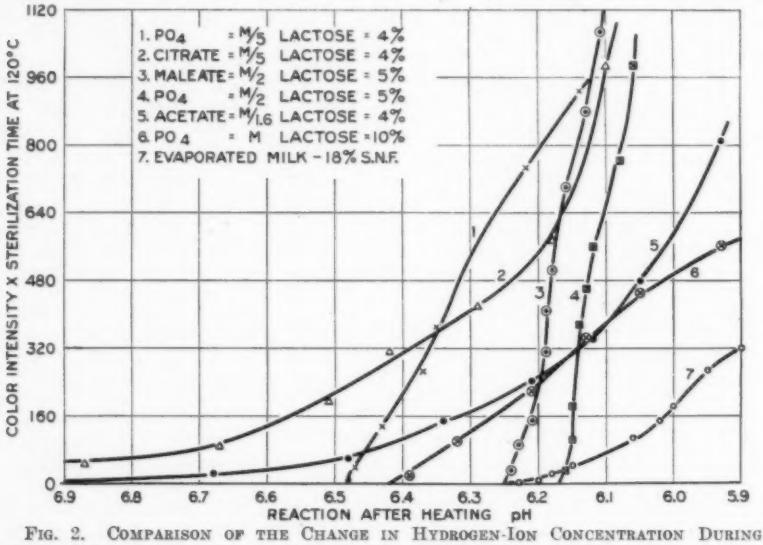

FIG. 2. COMPARISON OF THE CHANGE IN HYDROGEN-ION CONCENTRATION DURING HEATING WHICH OCCURS IN LACTOSE SOLUTIONS CONTAINING VARIOUS BUFFERS.

of a specific phosphate effect on reactions involving lactose. . . . Until it is shown that buffering acid radicals other than those present in milk but having practically the same dissociation constants, do not exert the same physical and chemical effects on the equilibria, we feel that any claims for specificity of action of phosphates in milk are unproved."

It appeared that a comparison of the color developed in lactose solutions with phosphate and maleate buffers would determine whether the phosphate itself was responsible for a catalytic influence when milk prod-

ucts are heated. Accordingly the data plotted in Fig. 3 were obtained comparing color development in these two solutions for different periods of heating. The curve for the phosphate solution shows the color of these samples to have been considerably darker than those containing maleate buffer.

Additional data were obtained to confirm the findings of Fig. 3. This

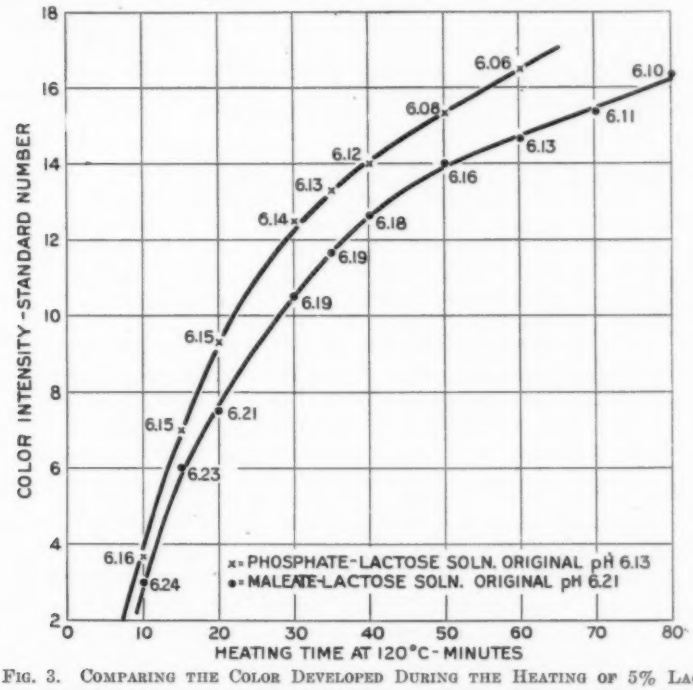

FIG. 3. COMPARING THE COLOR DEVELOPED DURING THE HEATING OF 5% LACTOSE SOLUTIONS IN M/2 PHOSPHATE AND M/2 MALEATE BUFFERS. THE FIGURES REFER TO THE pH AFTER HEATING. CONCENTRATION OF NH_4Cl CONSTANT AT M/23.

is reproduced in table 2 and again it is clearly indicated that phosphate exerts an effect favorable to increased color development during the heating of lactose solutions.

The amount of color formation promoted by phosphate-ions is dependent upon their concentration in the solution as shown by the data plotted in Fig. 4. The tubes containing the higher concentrations of phosphate showed much more color than those tubes having fewer phosphate-ions. The data was secured with and without NH_4Cl in the tubes.

Maleic acid is known to act as an anti-oxidant for fats and oils. A similar action in lactose solutions might produce an unduly light color and hence render a comparison between phosphate and maleate buffers of little

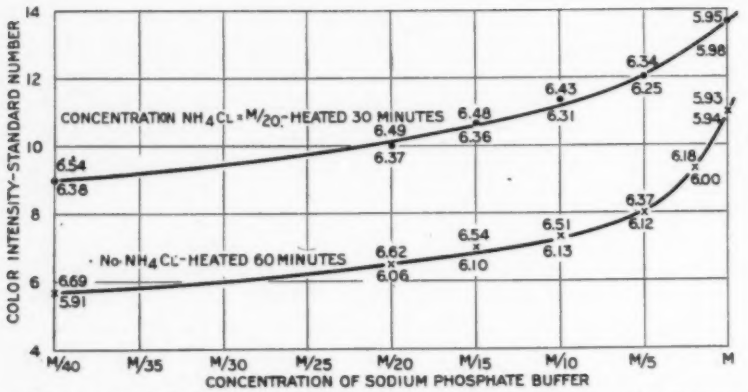

FIG. 4. THE INTENSITY OF COLOR DEVELOPED BY HEATING 5% LACTOSE SOLUTIONS AT 120° C. WITH AND WITHOUT NH_4Cl, USING PHOSPHATE BUFFERS OF DIFFERENT CONCENTRATIONS. THE UPPER FIGURES REFER TO THE pH OF EACH SOLUTION BEFORE HEATING, THE LOWER TO THE pH AFTER HEATING.

value. It did not seem probable however that sodium maleate would behave as an anti-oxidant in a non-fatty system such as a lactose solution. To be certain that the maleate buffer was not acting as an anti-oxidant in preventing color development and that mere traces of phosphate did not noticeably catalyze the reaction, the experiment reported in table 3 was carried out. Small quantities of either phosphate or maleate buffer in the presence of

TABLE 3

The effect upon the color of 5% lactose solutions of the presence of a small quantity of phosphate when maleate buffer is used during heating and conversely when phosphate is the chief buffer

Solutions heated to 120° C. for 30 minutes

PORTIONS OF M/2 BUFFER SOLUTIONS PER 6 CC. TOTAL SOLUTION. NH_4Cl CONSTANT AT M/20		REACTION		COLOR
MALEATE	PHOSPHATE	Before heating	After heating	
cc.	*cc.*	*pH*	*pH*	
6.0		6.22	6.20	10–⅔
5.9	0.1			10–⅔
5.5	0.5			10–⅔
1.0	5.0	6.15	6.11	12–⅔
0.5	5.5	6.12	6.10	12–⅔
0.1	5.9	6.12	6.12	12–⅔
	6.0	6.13	6.12	12–⅔

the other did not appreciably change the color of the solutions during heating.

The effect of lactose concentration upon color development was investigated using a maleate buffer and without the addition of an ammonium compound. The reaction was adjusted to simulate that encountered when milk is heated under like conditions. The results obtained are reproduced in table 4. It will be noted that a progressive increase in color follows the increases in lactose concentration.

TABLE 4

The relationship between lactose concentration and color development during heating. Maleate buffer concentration = M/2.

LACTOSE CONCENTRATION	REACTION		COLOR
	Before heating	After heating	After heating 120° C. for 30 minutes
Per cent	*pH*	*pH*	
2	6.55	6.51	5–⅔
4	6.56	6.44	7
6	6.56	6.41	7–⅔
8	6.54	6.37	8
10	6.56	6.34	8–⅓
12	6.55	6.32	8–⅔
15	6.53	6.26	9

A quantitative measurement of color development in buffered lactose solutions wherein the pH was held constant during heating was conducted, using different amino acids and ammonium compounds. The concentrations of these compounds were so adjusted that each tube of buffered lactose solution contained a concentration of ammonium or amino groups equal to M/70. In cases where this quantity of nitrogen compound produced little or no increase in color above the control, greater amounts were added. The results obtained are given in table 5. It will be seen that cystine, asparagine, the amides, and urea do not produce as much color as do the other compounds for a given quantity of NH_2. These results can perhaps be attributed to the extent to which these compounds enter into the reaction. The results of table 5 indicate that the darkening of the color of milk during heating is dependent upon the number of reactive amino groups which are present.

Different metals were added to lactose solutions before heating to study their effect upon color development. The data obtained in three different experiments are reproduced in table 6. Copper and iron caused a slight increase in color while tin showed a distinct tendency to lessen the color in-

TABLE 5

The effect of nitrogen-containing compounds upon color development in 5% lactose solutions buffered by M/2 sodium phosphate

CONCENTRATION OF COMPOUND IN LACTOSE SOLUTION		REACTION		COLOR AFTER HEATING TO 120° C.–30 MIN.
		Before heating	After heating	
		pH	*pH*	
Check—nothing added		6.17	6.16	7
Glycine	M/70	6.15	6.13	9⅓
Leucine	M/70	6.17	6.14	9
Cystine	M/70	6.16	6.19	7⅓
Tyrosine	M/70	6.16	6.15	9⅓
Asparagine	M/140			8
"	M/35	6.17	6.16	11⅔
Tryptophane	M/140	6.17	6.16	9
Acetamide	M/70	6.15	6.13	7
"	M/14	6.16	6.14	7
"	M/1.75	6.23	6.37	7⅔
Formamide	M/70			7⅔
"	M/17.5	6.17	6.14	9
Urea	M/140			7⅓
"	M/3.5	6.19	6.75	15⅔
$(NH_4)_2C_4H_5O_7$	M/210			9
$(NH_4)_2C_2O_4 \cdot H_2O$	M/140	6.16	6.13	9⅓
$(NH_4)Cl$	M/70	6.16	6.12	9⅓

tensity. These results are of special interest in the case of evaporated milk manufacture since this product is evaporated in copper vacuum pans but is sterilized and held in tin cans. The effect of metals upon color development during the sterilization of evaporated milk can probably be considered unimportant, with the inhibitive effect of the tin can counteracting the darkening influence of copper.

TABLE 6

*The effect of different metals upon the development of color in 5% lactose solutions heated to 120° C. for 20 minutes**

METAL	COLOR	METAL	COLOR
Not any	9⅔	Al	9⅔
Sn	8⅓	Pb	9⅔
Zn	9⅔	Hg	9⅔
Cu	10⅓	Ni	9⅔
Fe	10⅓	Monel	10

* Phosphate buffer concentration = M/2; $(NH_4)_2HPO_4$ concentration = M/42; reaction approximately constant at pH 6.18.

Solutions of 5 per cent lactose in M/2 phosphate buffer and containing a concentration of M/42 $(NH_4)_2HPO_4$ were sealed in atmospheres of dif-

ferent gases. After heating, the tubes were stored for several weeks. Some of the tubes were broken open to allow the air to freely reach the solution. The results obtained are shown in table 7. The differences in color of solutions heated in an atmosphere of air, nitrogen, oxygen, carbon dioxide or in a vacuum are very slight immediately after the heating period is completed. However, these differences in color are considerably intensified when the solutions are held in storage in the presence of air or oxygen. Evidently the development of color during storage either required, or is markedly increased by, the presence of oxygen.

TABLE 7

The effect of different gases upon color development in 5% lactose solutions heated to 120° C. for 20 minutes and stored at room temperature. Sodium phosphate buffer = M/2; $(NH_4)_2HPO_4 = M/42$. pH constant at 6.18

GASEOUS MEDIUM PRESENT IN TUBE		COLOR INCREASE DURING HEATING AND STORAGE			
DURING STERILIZATION	DURING STORAGE	COLOR AFTER HEATING	1 day	8 days	3 weeks
Air	Air—sealed	9⅔	10⅓	14	16+
Air	Air—open	9⅔	11⅓	14½	
Vacuum	Vacuum—sealed	9½	9½	9½	10
Vacuum	Air—open	9½	10⅓	14½	
Nitrogen	Nitrogen—sealed	9⅓	9⅓	9⅔	11
Nitrogen	Air—open	9⅓	10⅔	14½	
Oxygen	Oxygen—sealed	10⅓	11½	14½	16++
Carbon-dioxide	Carbon-dioxide—sealed	9½	9½	9½	10

The temperature of storage influences to some extent, the rapidity of color development after the initial period of high heating is over. Some results obtained on tubes of lactose solutions stored at 10° C., 25° C., and 42° C., after sealing in air and heating, are given in table 8. The higher temperatures of storage caused greater darkening in color.

It was interesting to note that the color which developed in heated lactose solutions during storage was markedly browner in hue than that in solutions in which the same depth of color was developed by heating alone. This change in shade from reddish yellow to brown occurred largely during the first few days of storage. It was difficult to match the stored samples with the color standards used for the freshly heated solutions but the results given for these samples correctly reflect the order of color intensity in each, although the match with the standard was not perfect.

In view of the foregoing results, it did not appear that any normal variation in the manufacturing process of condensed or evaporated milk would

TABLE 8

The development of color during storage of 5% lactose solutions buffered by M/2 sodium phosphate and heated to 120° C. for 20 minutes. Average initial reaction = pH 6.12; average final reaction after heating = pH 6.16

STORAGE TEMP.	CONCN. OF $(NH_4)_2HPO_4$	COLOR DEVELOPMENT DURING STORAGE					
		Not held	1 day	4 days	12 days	26 days	60 days
° C.							
10		5	6	7⅓	7⅔	7⅔	7⅔
25		5	6	7⅔	7⅔	8	9
42		5	6	7	8	9⅓	11
10	M/42	9⅔	10⅔	11⅓	13	15	15½
25	"	9⅔	11	13	14½	16	18*
42	"	9⅔	11	13	14½	17*	20*

* Approximate figures. No color standards available darker than 16.

eliminate color development, but it was deemed of interest to know what chemical means might be effective in preventing the appearance of color in lactose solutions during heating. A number of compounds were added to 5 per cent lactose solutions buffered by M/2 sodium phosphate and containing M/20 $(NH_4)_2HPO_4$. Small quantities of hydroquinone, β-naphthol, resorcinol, catechol, and chromous chloride did not prevent the development of the usual amount of color shown by control samples heated under identical conditions.

Sodium bisulfite was very effective in preventing the development of any brown color in milk or lactose solutions during heating. At a concentration of M/24 it prevented any color development in the above lactose solution after heating to 120° C. for 20 minutes. The action of sulfite in retarding color formation in sugar syrups is well known in the cane and beet sugar industries.

Ramsey, Tracy and Ruehe (8) have observed that color formation in evaporated milk could be prevented when sufficient formaldehyde was added before sterilization. In view of the known affinity of formaldehyde for amino acids, this observation was taken as further evidence of a sugar-amino condensation reaction being entirely responsible for color formation. Some interesting data on the effect of increasing quantities of formaldehyde upon color development in lactose solutions are given in table 9. The experiments were run with and without additions of glycine to the solutions. Very small concentrations of formaldehyde markedly increase the color of the solutions while larger quantities inhibit color formation. The trend of the results appear the same whether glycine is present or absent. The action of formaldehyde appears to be more or less independent of the

TABLE 9

The effect of formaldehyde upon the color of 5% lactose solutions heated to 120° C. for 30 minutes. Half molal concentration of maleate buffer in all solutions

FORMALDEHYDE CONCENTRATION	NO GLYCINE		COLOR	GLYCINE CONCENTRATION = M/142		COLOR
	Reaction			Reaction		
	Before heating	After heating		Before heating	After heating	
%	*pH*	*pH*		*pH*	*pH*	
0.00	6.64	6.44	7½	6.66	6.44	10½
0.14		6.45	10		6.44	15
0.35		6.43	9		6.44	14
0.57		6.41	8⅓	6.66	6.40	13⅔
1.14		6.40	5½	6.65	6.37	11
1.60	6.67	6.40	3½	6.62	6.36	10
2.00	6.66	6.34	1½	6.58	6.29	8

amino acid content of the lactose solutions. Substantially the same results as those reported in table 9 were obtained by using sodium phosphate as a buffer in place of maleate buffer. The nature of the reactions involved here are too obscure to render attempts at an explanation profitable at this time.

The same general relations found to exist for color development in lactose solutions were found to hold true also for color formation in skim milk. However, due perhaps to the opaque nature of the milk, many of the delicate differences which are easily seen in the lactose solutions are not apparent in the milk. Pure solutions are therefore much superior to the heterogeneous mixture found in milk for a study of the basic relationships of the different components.

DISCUSSION

Since the hydrogen-ion concentration of a lactose solution is of great importance in color development during heating, a study of color formation must be planned to include adequate control of the reaction. From the results reported here it would appear that sodium phosphate and sodium maleate are very satisfactory buffers for maintaining the pH of a solution near to that found in milk, the phosphate radical being normally present in milk and the maleate being available for use where milk constituents are to be excluded from the solution.

The study of color development in the presence of phosphate and maleate buffers showed in three different series of experiments that the phosphate possessed a specific effect in darkening the color of heated lactose solutions. That portion of the darkening which could be attributed to

phosphate ions required the presence of appreciable quantities of phosphate, the depth of color developed being dependent upon the quantity of phosphate ions in the solution.

While it is interesting to speculate as to the nature of the reactions involved in the color development noted in this paper, there is insufficient evidence at hand to formulate any reaction mechanism. The data of table 4 show that color will form in pure lactose solutions during heating in the absence of all other materials except a maleate or phosphate buffer. This fact indicates that part of the color developed in milk during heating is due to a caramelization of the lactose.

Considering the large amount of research which has been done by many investigators it appears certain that there is a combination between amino acids and reducing sugars. The data given here show that the presence of an ammonium salt or an amino acid causes a very great darkening in the color of heated lactose solutions. Because of the limited variation in the pH of milk, and its relatively high protein content, this amino-sugar combination doubtless accounts for a major portion of the color formed in milk during heating. Since the phosphate ion concentration was found to influence color in lactose solutions and since the concentration of this ion is relatively high in milk, the contribution of the phosphate to color development is probably considerable.

The findings that the shade of color produced in storage was different from that which is formed during heating seem to indicate that two different reactions occur. Additional evidence to this effect has previously been shown in the case of evaporated milk (15) which was sterilized and held in storage. During sterilization there was a progressive change in hue, brilliance and chroma while the color change observed during storage was one in chroma only.

Results from the study of color development in lactose solutions seem to justify certain conclusions with regard to evaporated milk. Perhaps half of the color of evaporated milk as it reaches the consumer is developed during storage, the other half arising as a result of the sterilization process. The color which forms during storage may be partially or almost wholly eliminated, depending upon how brief the storage period can be made. If storage is necessary temperatures of 15° C. or lower are desirable. Where the storage temperature is not controlled, summer storage is much more detrimental to the color of the product than is storage for the same length of time during the winter.

Improvement in the color which appears during sterilization of evaporated milk is more difficult. Complete elimination of oxygen from the milk before sterilization should yield a lighter product. However the degree of improvement would probably not justify the expense of this procedure.

The addition of chemical substances which might prevent color development would of course not be permissible.

Exclusive of hydrogen-ion concentration, the lactose content of the milk and the composition of the gases present in the container, the factors which contribute to the color of milk during heating may, from the results of this investigation, be listed approximately in the order of their importance as: (1) reactive amino groups contained in the protein or arising as protein decomposition products, (2) the normal tendency of the lactose on heating at pH 6.5, to form brown lacto-caramel, (3) the phosphate content of the milk, and (4) the presence of metallic catalysts.

SUMMARY

1. Reproducible color standards for measuring the color of lactose solutions in which heat has produced varying shades of brown have been described and defined in numerical terms according to the Munsell system of color measurement.

2. The presence of the phosphate radical in lactose solutions during heating has been shown to exert a specific effect in causing darkening in the color of these solutions.

3. Color development in lactose solutions during heating is increased with increasing concentration of hydroxyl-ions, lactose, amino acids, ammonium salts, phosphate and oxygen. The presence of copper or iron catalyzes the color reaction while tin retards color formation. A very small quantity of formaldehyde increases color while larger amounts markedly restrict color development. Sodium bisulfite will entirely prevent the appearance of color. When amino acids or proteins are present during heating, color is probably due both to the formation of a complex material formed from the lactose and an amino group and to a polymerization of the sugar to lacto-caramel. Either reaction may occur at the hydrogen-ion concentration found in milk.

4. An effective means of preventing color development in lactose solutions during heating which would be suitable for use in improving the color which appears in evaporated milk during sterilization was not found. However, the results obtained with lactose solutions substantiate the fact that the objectionable darkening in color of evaporated milk which occurs during storage can be materially lessened by shortening the storage period or lowering storage temperature.

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THE USE OF CITRIC ACID AND SODIUM CITRATE IN BUTTERMAKING

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The flavor and aroma of butter have always been recognized as being of the utmost importance in determining the quality of butter. Many of the larger markets demand a butter with a high flavor and aroma and to meet this demand it is necessary to make the butter from ripened cream. The acidity of such butter may be the cause of loss to the manufacturer or wholesaler due to the fact that such butter deteriorates more rapidly in storage.

During the past few years there has been considerable research carried on in an effort to isolate and identify the substances that are responsible for the flavor and aroma of butter, in order that these aroma constituents might be added in pure form to butter made from sweet cream and thereby give the butter the high flavor and aroma characteristic of ripened cream butter, without the possible danger of deterioration due to the high acidity. All of the investigators seem to agree that the major flavor and aroma constituents are methylacetylcarbinol and its oxidation product diacetyl. The amount of these constituents present in the butter varies somewhat according to the different investigators; Davies[1] reporting 0.05 to 0.5 parts per million, Obst[2] found five grams of diacetyl in 2000 pounds of butter or 5.5 parts per million, while Darres[3] reports that the presence of 0.000,000,2 per cent of diacetyl gives butter its characteristic aroma.

Pritzker[4] reports that wood vinegar is a very cheap source of these compounds as he found diacetyl in amounts varying from 0.1 to 1.92 grams per liter, and methylacetylcarbinol 0.5 to 12.0 grams per liter. Numerous other sources have been studied and these aroma constituents of butter have been found in varying amounts in such substances as coffee, cacao, and honey.[5]

As a result of the isolation of these flavor constituents of butter, a num-

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[1] Davies, W. L., Food Manufacture 8: 346–8 (1933); *cf.* Chem. Abs. 28: 835, (1934).

[2] Obst, W., Molk.-Ztg. Hildesheim No. 64, June 5, 1930, pg. 193–4; *cf.* Milchw. Literaturb. 3: 367, 1930.

[3] Darres, W. C., Sonderdruck aus Ford Manfacture Okt. 1933, 35; *cf.* Milchw. Literaturb. 3: 367, 1930.

[4] Pritzker, J., Chemiker-Ztg. No. 80, Oct. 7, 1933; *cf.* Milchw. Literaturb. No. 81, Feb. 28, 1934.

[5] Schmallfuss and Barthmeyer, Biochem. Zeitsch. 216: 330–5, 1929; *cf.* Chem. Abs. 24: 1432 (1930).

ber of artificial butter flavors have appeared on the market in an attempt to meet the demand for a butter with a high aroma and a low acidity. Such additions have been tried in some of the European countries and the general opinion of the writers is that while there is a marked improvement in the flavor and aroma of the butter, the effect is rather temporary and the diacetyl and methylacetylcarbinol may act as catalysts in hastening the deterioration of the butter that is not used within a few days after the addition of the artificial flavoring material. Another objection that is raised is that such materials may be used in margarine in an attempt to give a more exact imitation of butter.

In the isolation of these flavor constituents the first materials to be studied were the starter cultures used in the manufacture of butters with decided aromas and flavors. While the amount of material isolated from this source was naturally very small, the figures given previously indicate that only a very slight trace of diacetyl is needed to increase the aroma of butter. There is a difference of opinion as to the source of these aroma constituents, as indicated by the writings of several investigators. Cuisa[6] decided that the methylacetylcarbinol was formed through the action of trypsin and its enzymes on the proteins. Davies[7] states the carbinol is formed from the lactose, certainly the latter is more easily acted upon by the bacteria in the butter starter. Hammer and his associates[8] report a detailed study on the presence of methylacetylcarbinol and diacetyl in starter cultures. They show that the amount of these constituents in a culture is increased by the addition of citric acid or sodium citrate in varying amounts. Raffey[9] reports similar results using 0.1 to 0.4 per cent citric acid in the milk. In a recent paper Ritter and Strussi[10] have confirmed the above findings that the aroma of butter is due to the presence of diacetyl which is formed by the aroma producing organisms through the utilization of the citric acid. These authors also added citric acid to the cream, but in their opinion this addition resulted in the production of excessive flavor.

The addition of citric acid and sodium citrate to starter cultures was investigated by Templeton and Sommer.[11] It was found that the addition of 0.2 per cent of citric acid or its equivalent as sodium citrate to the milk to be used in the starter culture increased the volatile acidity approximately 50 per cent, while the titratable acidity increased only 10 per cent. While there is no direct evidence that the volatile acids are responsible for the

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[7] Davies, W. L., Food Manufacture **8**: 346–8 (1933); *cf.* Chem. Abs. **28**: 835 (1934).

[8] Hammer, B. W., Michaelian, M. B., and Farmer, R. S., Iowa State College of Agriculture Research Bulletin No. 155.

[9] Raffey, O., Osterr. Milchwirtschaft. Ztg. **20**: 250–1 (1932); *cf.* Chem. Abs. **27**: 2735 (1933).

[10] Ritter, W., and Strussi, D., Schweiz. Milchztg. **60**: 47–8 (1934); *cf.* Chem. Abs. **26**: 4494 (1934).

[11] Templeton, H. L., and Sommer, H. H., Jr. Dairy Science **12**: page 21, 1929.

characteristic flavor and aroma of butter or starter cultures, they no doubt play some part either as intermediates or in establishing conditions which favor the growth of the aroma producing organisms. There is evidence that high volatile acidity may be indicative of a high diacetyl and methylacetylcarbinol content of the starter culture and the butter made from such a starter.

These findings indicate quite definitely that the flavor and aroma of butter may be increased by the enrichment of the citrate content in the starter or cream. The fact that milk is known to be quite variable in its citrate content may be regarded as further justification for such a practice. This practice of supplementing the natural composition of the milk is now definitely established in the evaporated milk industry, where it is frequently necessary to add citrate or phosphate to control the stability.

The results of the researches to be presented in this paper are a continuation of the previous work, since the starters studied earlier were used in the making of the butter to be described at this time. These were regular commercial starter cultures propagated in both sterilized and pasteurized milk for more than two months before the buttermaking studies were started. Eight of these cultures were used, and a series of experiments were performed with each one using different treatments of the cream and starter, as will be given in detail in connection with the tables. In each series of experiments there was one sample of butter that was made with the culture alone, without the addition of either citric acid or sodium citrate to the culture at any time. This was the control sample for each series of experiments.

The butter made in this study was scored as fresh butter within ten days after making, again after six weeks' storage at 40° F. (4.40° C.), and after six months' storage in an ice cream hardening room where the average temperature was approximately 0ö F. (−17.78° C.). The butter was packed in carefully cleaned half-pint Mason jars and three jars were filled from each churning to insure an unused sample for each scoring. The men requested to score the butter were competent judges, and the samples were numbered in such manner that the same treatment did not always have the same number. In some cases the judges did their scoring as a group, while other scorings were made by each judge on different days. Sixty-five samples of butter were made on a laboratory scale using a small hand churn of the kangaroo type. The butter was worked in a small home-made worker equipped with rolls similar to those found in large churns. In order to insure as uniform a body as possible, the butter was all churned at approximately the same temperature and received the same amount of working. Two per cent of salt was added to the butter before working. As indicated previously, the butter was packed in Mason jars and these in turn were kept in cardboard boxes to exclude light.

In table No. 1 the capital letters indicate the starter that was used. The

cream used in this series of experiments was taken directly from the separator, pasteurized at 140° to 145° F. (60.00° to 62.78° C.) for 30 minutes and then cooled rapidly to 70° F. (21.11° C.). At this temperature the cream was innoculated with the starter using about 5 per cent, and the citric acid or sodium citrate added in amounts indicated. After thorough mixing of the sample the titratable acidity was determined and all samples having 0.35 per cent or more calculated as lactic acid were immediately cooled to 45° to 50° F. (7.22° to 10.00° C.) and held at this temperature until churned. The remaining samples were kept in a water bath at 70° F. until the titratable acidity was .35 per cent. This holding time varied from six to ten hours although a few samples required a somewhat longer time. The cream was chilled for at least four hours before churning, the maximum time being twelve hours. In the series of experiments marked with a single letter, the cream used tested about 30 per cent butterfat, and four and one-half pounds of cream were used for each sample.

In those series of experiments marked with the double letters, the starter used was the same as in the corresponding single letter, but only one liter of 55 to 60 per cent cream was used for each experimental churning. This cream received the same treatment as the other cream except that when the cream had been cooled to 70° F., 750 cc. of the starter culture were added to each liter of cream with the other additions as indicated in table 1a. The cream and starter were thoroughly mixed by shaking. As the titratable acidity in each case was over 0.35 per cent the samples were immediately cooled to 50° F. and placed in storage. These samples were scored at the same ages and by the same judges as the other samples.

The explanation of the terms used in the treatment of the different samples is the same as in the paper on starters, namely—fresh citric acid or sodium citrate indicates that the additions were made to a control culture 24 hours previous to the inoculation of the sample of cream; an old citric acid or sodium citrate culture is one that has been carried through a number of transfers, with citric acid or sodium citrate added to the culture at each transfer before the inoculation of the sample of cream. When the term "control" is used, that indicates that the starter added did not contain any added citric acid or sodium citrate.

In tables No. 1 and 1a, the average scores of each sample are given at the three scoring ages. It would be impossible to present the individual scores and the comments of the judges on all the samples of butter, so these summary tables are presented. In the series with starter culture "A" two sample jars were broken after the first scoring so that the six weeks' scoring was omitted in two instances. Table No. 2 is based on the preceding tables, in that the samples of butter are ranked in order of their scores within the series with each starter culture, and these rankings are compiled as shown. Tie scores were broken in favor of the sample having the highest score from one of the judges, or if that were impossible on the basis of the criticisms

Average scores of butters made with additions of citric acid and sodium citrate

TABLE I

TREATMENTS	A			B			C			D			E			F			G			H			AVERAGES		
	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.
Control	91.13		90.75	91.13	89.67	89.63	91.25	91.00	89.38	91.38	91.33	89.25	91.50	91.33	89.31	91.25	91.50	90.88	91.25	90.00	89.13	91.00	89.17	88.13	91.23 (6)*	90.57 (7)*	89.40 (8)*
Control + .25 Citric Acid				90.88	89.50	88.88	91.75	91.25	90.31	91.44	91.00	89.25	91.13	91.00	89.75				91.38	91.50	89.63	91.63	91.00	89.94	91.57 (6)	90.60	89.79
Control + .45 Citric Acid							90.38	89.17	88.63							91.13	90.33	90.63				90.75	88.50	87.88	90.75 (3)	89.34	89.05
Fresh Citric Acid Culture	92.38	91.50	89.50	91.25	89.17	90.13	90.75	90.50	90.06	91.00	91.67	90.25	90.88	91.67	91.13										91.25 (6)	90.90	90.16
Fresh Citric Acid + .15 Citric Acid																			92.06	91.17	90.94	91.50	90.17	88.07	91.78 (8)	90.67	90.00
Fresh Citric Acid + .25 Citric Acid																91.63	92.17	91.00							91.63 (1)	92.17	91.00
Old Citric Acid + .15 Citric Acid													92.38	92.42	89.88				91.50	91.17	91.00				91.94 (2)	91.79	90.44
Old Citric Acid + .25 Citric Acid	90.75	90.17	90.63	91.19	89.25	90.00				91.14	91.75	89.81													91.02 (3)	90.42	90.15
Fresh Sodium Citrate Culture							90.88	90.00	89.38	91.38	92.25	90.81	90.75	91.83	91.69										91.00 (2)	91.36	90.63
Fresh Sodium Citrate Culture + .15 Sodium Citrate	91.88	91.00	90.88													91.50	91.00	90.25							91.69 (4)	91.00 (3)	90.57 (4)
Fresh Sodium Citrate Culture + .25 Sodium Citrate	91.25		90.81	91.25	89.88	90.75													91.13	90.67	90.06	91.50	91.00	89.38	91.38 (1)	90.80 (2)	90.25
Old Sodium Citrate Culture																91.25	92.75	91.94							91.25 (2)	92.75	91.94
Old Sodium Citrate Culture + .25 Sodium Citrate																			91.50	90.67	90.31	91.19	91.00	89.88	91.31 (6)	91.28	90.10
Old Sodium Citrate Culture + .25 Sodium Citrate	91.69	90.75	91.00	91.13	90.25	90.69	92.13	91.83	89.75	91.13	91.42	90.25	91.38	90.33	89.63	91.63	92.50	90.50							91.61 (6)	91.19	90.30
AVERAGE	91.50	90.96	90.69	91.14	89.64	90.18	91.19	90.64	89.50	91.24	91.57	89.96	91.39	91.43	90.33	91.40	91.71	90.57	91.47	90.86	90.18	91.25	90.88	89.81	91.32	90.67	90.10

* Figures in brackets indicate number of scores averaged

Average scores of butters made with additions of citric acid and sodium citrate

SERIES	AA			EE			BB			AVERAGE		
TREATMENTS	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.
Control	91.25	90.00	90.44	91.88	91.33	89.63	91.38	91.63	90.50+	91.50	91.06	90.19
Control + .25 Citric Acid	92.13	89.88	89.63	91.25	91.00	89.75	91.25	92.06	91.19	91.54	90.97	90.19
Control + .45 Citric Acid	92.13	90.00	89.50	92.13	92.50	89.69	92.00	91.50	90.69	92.09	91.36	89.96
Old Citric Acid + .25 Citric Acid	90.63	90.25	90.06	91.25	90.25	90.50				90.94	90.25	90.28
Control + .25 Sodium Citrate	91.13	90.75	89.00							91.13	90.75	89.00
Control + .25 Sodium Citrate .25 Citric Acid							92.81	92.88	92.00	92.81	92.88	92.00
Old Sodium Citrate + .25 Sodium Citrate	91.25	90.19	90.13	91.63	91.67	90.38	92.25	92.06	90.88	91.71	91.31	90.46
Old Sodium Citrate + .45 Sodium Citrate				91.63	92.00	90.38	91.88	92.00	90.25	91.75	92.00	90.31
	91.42	90.18	89.79	91.63	91.47	90.08	91.93	92.07	90.92	91.66	91.24	90.25

given. From this table it is evident that in the single letter series there were two or more samples that were preferred to the control sample in all but one instance. The column at the right of this table gives for each treatment the percentage of samples that ranked third or better. In the double letter series there were two instances in which the control sample was either first or second, and it was below third in all the others.

On the basis of the first two scorings of this butter it seemed advisable to do further work on a larger scale. The second series of experiments was conducted on cream arriving at the University creamery at a time when the amount of cream varied rather widely from day to day and the acidity was usually rather high. Most of the cream was neutralized to 0.25 per cent acidity with a commercial sodium bicarbonate neutralizer before pasteurization. When there was sufficient cream it was divided into two portions. After cooling to 70° F. one vat was inoculated with the starter culture to act as the control, and citric acid or sodium citrate added to the other in the amounts shown in table No. 3. The butter was made by the regular buttermaker under the conditions usually existing in the plant. When the butter was removed from the churn three half-pint Mason jars were filled as samples for the judges to score. The samples were scored as fresh butter and again after six weeks and six months. The first five lines of table No. 3 represent a portion of the results obtained in this second series of experiments. All of these were made from starter culture "E" except the one in which the citric acid was added to the cream without starter. The average scores of these samples by the same judges are given in the table. In connection with this series of experiments the fat losses in the buttermilk were studied, using the Butyl alcohol modification of the Babcock test. The results showed that the treatment received by the cream did not increase the fat losses in the buttermilk. In some instances there was a decided reduction in the fat loss.

As a further confirmation of these results, another series of commercial churnings were made and two samples of each were submitted to the same

TABLE I

SCORING OF BUTTER SAMPLES*

TREATMENT	FIRST			SECOND			THIRD			FOURTH			FIFTH			SIXTH			% Above Fourth Place
	Fresh	6 Mos.	6 Mo.	Fresh	6 Mos.	6 Mo.	Fresh	6 Mos.	6 Mo.	Fresh	6 Mos.	6 Mo.	Fresh	6 Mos.	6 Mo.	Fresh	6 Mos.	6 Mo.	
Control # 1				1			2	2	1	1	2	3	4	2	1		1	3	26
Control + .25 Citric Acid	2	1	2	1	1			1		2	1	1		1	2	1	1		44
Control + .45 Citric Acid												1				3	3	2	69
Fresh Citric Acid Culture	1	1		1		2		1	2		2		2			1	1	1	58
Fresh Citric Acid + .15 Citric Acid	1			1	1	1					1				1				67
Fresh Citric Acid + .25 Citric Acid				1		1		1											100
Old Citric Acid + .15 Citric Acid	1	1	1				1	1	1										100
Old Citric Acid + .25 Citric Acid					1		1			1	1	2		1	1	1			92
Fresh Sodium Citrate Culture		1	2	1	1					1		1		1		1			58
Fresh Sodium Citrate Culture + .15 Sodium Citrate				1	1	1	1							1				1	67
Fresh Sodium Citrate Culture + .25 Sodium Citrate	1		1		2		1		2	1	1	1							67
Old Sodium Citrate Culture		1	1							1									67
Old Sodium Citrate Culture + .15 Sodium Citrate		1		1		1			1	1				1					67
Old Sodium Citrate Culture + .25 Sodium Citrate	2	2	1		1	2	2	1	1		1		2		2		1		67

Control # 2 a			1	1						1	1		1	2	1			1	72
Control + .25 Citric Acid	1					1		1				2	1	1		1	1		83
Control + .45 Citric Acid	1	1		1			1				1	1			2		1		44
Old Citric Acid + .25 Citric Acid			1		1				1							2	1		80
Control + .25 Sodium Citrate		1											1					1	35
Control + .25 Sodium Citrate + .25 Citric Acid	1	1	1																100
Old Sodium Citrate + .25 Sodium Citrate				1	1	2	1	2	1	1									80
Old Sodium Citrate + .25 Sodium Citrate					1		1		1	1	1							1	80

* Each treatment (sample) was a member of a series of six samples. Each treatment was represented several times (see Tables I and Ia) in such six sample series, according to the number of original starter cultures with which the treatment was tried. At each scoring each treatment could rank anywhere from first to sixth place. The table records the number of times the respective treatments ranked first, second, third, fourth, fifth and sixth. The final column is explained by the following illustration:—Control No. 1 is represented 23 times in first to sixth rank, but only six times in the first three places. Therefore, it placed third or better in only 26 per cent of the placings.

judges that had scored the others, as well as to a number of other men in different parts of the country. These samples were scored as fresh butter and again after six months storage. As this work was done in August and the butter had to be shipped some distance in small cardboard boxes, it is possible that the score that the butter received might have been higher if shipping conditions had been better. The scores of these samples of butter were often times very divergent, ranging from 87 to 92 on samples of butter from the same churning. The last three lines of table No. 3 give the aver-

TABLE III

Average scores of butters made with additions of citric acid and sodium citrate

CONTROL BUTTER SCORES			TREATED BUTTER SCORES			TREATMENT
Fresh	6 wk.	6 mo.	Fresh	6 wk.	6 mo.	
91.75	90.58	90.67	92.08	91.00	91.50	Fresh Citric Acid Culture
			89.67	90.00	90.33	Fresh Sodium Citrate Culture
91.83	90.08	90.33	91.08	89.50	89.33	No starter + .075% Citric Acid Added
91.83	89.75	88.83	90.96	90.00	90.33	Control + .1% Citric Acid as Sodium Citrate
			91.46	90.25	90.67	Control + .1% Citric Acid
90.63		89.91	90.67		89.87	Fresh Citric Acid Culture
89.79		88.08	89.90		89.88	Control + .1% Citric Acid as Sodium Citrate
91.19		89.42	90.03		88.96	Old Sodium Citrate Culture + .1% Citric Acid as Sodium Citrate

age scores of the eleven men examining these samples of butter. The first two samples were made with starter culture "E" and the last one with "H."

While the results of these later series of experiments do not show the same degree of improvement of the treated samples over the controls there is evidence that the addition of citric acid or sodium citrate to the starter culture, the cream to be used for the buttermaking, or both, gives the resulting butter higher flavor and aroma.

SUMMARY

This work shows that the addition of citric acid or sodium citrate to either cream or the starter or both tends to produce a butter of more desirable flavor and aroma than the untreated butter made from the same cream.

The findings of previous investigators concerning the effect of high acidity on the keeping quality has been confirmed in some of the samples of butter scored, while in other instances there is very little difference in the scores of the fresh butter and the score after storage. In numerous samples the score after six weeks' storage was higher than that given the fresh butter.

It was noticed that the use of citric acid tended to lower the fat losses in the buttermilk.

In flavor and aroma the buttermilk from the treated cream compared very favorably with the starters.

The addition of citric acid or its equivalent in sodium citrate in amounts not to exceed 0.2 per cent of the weight of the cream is sufficient to produce an increase in the score of the butter made from the cream. Their addition in the starter only, is noticeable in the score of the butter.

In conclusion the authors wish to express their thanks to Charles Pfizer and Company, Inc., for the fellowship that has made this work possible, and to those gentlemen who gave their time and services in the scoring of the samples of butter.

THE ACTION OF MILK FAT AS A FOAM DEPRESSANT

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The action of lipoid substances as foam deterrents is a well-known phenomenon. Eichholtz (1) concluded that the action of these substances was due to the removal by them of the protein adsorbed at the air-solution interface. Van Dam (2) attributed the destructive action on foam of the fat globules in milk to the distortion produced in a thin film when the thickness of the film approached the dimensions of the globules. Sommer and Horral (3) assumed that the reduction in foaming power occurred because the serum did not adhere to the fat globules with a force equal to the cohesion of the serum for itself, as a consequence of which a point of weakness existed in the neighborhood of the fat globules. King (4) recently observed by means of a microscope the changes occurring in skim milk foam when the fat contained in whole milk is permitted to act on it. He suggested as a possible explanation of the observed changes the lowering in surface tension due to the formation of a fat film. Reference was made in the publication to the work of Mohr and Broekman (5) who observed a lowering in superficial viscosity corresponding to an increase in the fat content and to a decrease in the foaming power of milk.

The results of investigations in this laboratory on surface phenomena in milk products are concerned in part with the views expressed by the various authors quoted above. These results will be presented and discussed in this paper.

Experimental

In the following experiments the surface properties of milk serum prepared from skim milk by ultrafiltration were compared with the surface properties of milk serum containing one per cent by volume of added skim milk. The addition of the skim milk to its serum produces a marked decrease in the foaming power of the serum, an effect which may be demonstrated to be due to the minute lipid content of the resulting suspension.

The following measurements were made:

(1) Foaming power
(2) Surface tension (dynamic and static)
(3) Superficial viscosity
(4) Adsorption of nitrogenous material

Measurement of Foaming Power: To measure foaming power, 20-cc. samples were measured into graduate cylinders and agitated, uniformly, in

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pairs to give relative values for the quantity of foam remaining one minute after agitation has ceased. Typical results obtained by means of this procedure are included in table 1.

TABLE 1

The dynamic and static surface tension values of water and milk serum solutions. The relative foaming capacities of these solutions

SYSTEM	SOLUTION	RELATIVE FOAMING CAPACITIES	DYNAMIC VALUES (JET METHOD)	DYNAMIC VALUES (RING METHOD)	STATIC VALUES (RING METHOD)
			dynes/cm.	*dynes/cm.*	*dynes/cm.*
Serum	Water		72.0	72.0	72.2
	Milk serum	1.0	72.0	61.3	51.1
	Milk serum +1% skim milk	0.5	72.0	61.3	50.7

Measurement of Surface Tension: Static surface tension measurements were made by the ring method. Dynamic measurements were made according to the method of capillary jets, as described by Rayleigh (6) and according to a procedure based on the ring method and differing from it only in that the surface of the solution under investigation was continuously renewed during a measurement by the slow and controlled overflow at the surface of the solution.

The measurement by the method of capillary jets furnished information concerning the tension of surfaces 1/200 second old, while the modified ring method permitted the measurements of dynamic tension corresponding to a somewhat older surface.

Typical results are given in table 1. The values given for the dynamic surface tension as determined by the method of capillary jets represent merely those calculated relative to that of water on the basis that the tensions vary inversely as the square of the wave-length of the jets.

The differences in the jet characteristics studied bear no correspondence to differences in foaming power. Thus, both milk serum and the serum containing skim milk show the same dynamic surface tension as pure water. Neither the dynamic, nor the static values as determined by the ring method show any significant differences in the tensions of the two solutions. Consequently, contrary to the ideas expressed by King, it is hardly likely that the action of the fat in milk as a foam deterrent is to be correlated with its action as a surface tension depressant.

Measurement of Superficial Viscosity: Superficial viscosity determinations by the method depending upon observations of the damping of a torsional pendulum yielded values for milk serum, and for the serum containing skim milk, which were much greater than the value for pure water. No significant difference, however, existed between the surface viscosities of the

two solutions. The data pertaining to these measurements are omitted. It was found difficult to obtain reproducible results by this method, probably because of difficulty in the production of surfaces of the same age. In order to avoid this difficulty, a procedure was developed depending upon the impedance offered to the rise of small bubbles through a slightly inclined tube containing the solution under investigation. The ratio between the time required for a bubble to travel one centimeter, in the solution under consideration, and the time required for a bubble of the same size to traverse the same distance in a sucrose solution of the same bulk viscosity and of approximately the same density as that of the solution under investigation, was arbitrarily taken to represent an index of the magnitude of viscous forces localized on the surface of the bubble. The indices obtained in this manner are given in table 2. The size of the bubble, and the bulk viscosity, and density of the sucrose solution require control, inasmuch as they are factors influencing the rate at which the bubble rises.

TABLE 2

The viscosity and superficial viscosity indices of various reference solutions, and of milk serum solutions, as determined by observations of the rate of rise of small bubbles

SYSTEM	SOLUTION	RADIUS OF BUBBLE	VELOCITY OF BUBBLE	SUPERFICIAL VISCOSITY INDEX	RELATIVE VISCOSITY 30° C. $H_2O = 100$
		cm.	*cm./sec.*		
Reference	Water	0.25	4.31		1.00
Solutions	7% sucrose	0.25	3.30		1.16
	11% "	0.25	2.90		1.32
	20% "	0.25	2.50		1.88
	30% "	0.25	1.88		2.98
	40% "	0.25	1.17		5.48
Serum	Milk serum	0.30	1.41	2.34	1.16
	Milk serum + 1% skim milk	0.30	1.71	1.93	1.16
	Serum + 2% skim milk	0.31	1.84	1.79	1.16

The results indicate that the addition of skim milk to milk serum produces a decrease in the superficial viscosity index of the serum. This change may possibly be due to the lubricating action of the fat. However, it is doubtful whether this decrease in the superficial viscosity index is sufficient to account for the marked differences observed in foaming power inasmuch as the superficial viscosity of the serum, even after the addition of milk, is quite marked. Supporting this conclusion is the observation (date not recorded in this paper) that the superficial viscosity of a dilute sodium oleate solution of marked foaming power is the same as that of a suspension

of weak foaming power, containing free oleic acid in addition to sodium oleate.

In contradistinction to the results of superficial viscosity measurements by the observation of the damping of a torsional pendulum, the results of superficial viscosity determinations on sodium oleate solutions by the method described above indicate the existence of a viscous force considerably greater than that belonging to pure water.

Adsorption Determinations: The method used to measure adsorption was similar to that employed by McBain (8) and his collaborators. Bubbles uniform in size of measured volume and area were removed after their passage through a slightly inclined tube containing the solution under investigation; and from determinations of the weight of the solution resulting from the collapse of the bubbles, and of the concentration of the nitrogenous material in this solution, in the original solution, and in the residual solution, the quantity of nitrogenous material adsorbed per square centimeter of surface was calculated.

Nitrogen determinations were made by means of the Arnold-Gunning modification of the Kjehldahl method. All samples were run in duplicate. Distillation was effected by means of steam according to the method described by Allen in which the essential features of the micro-Kjehldahl apparatus are utilized for ordinary Kjehdahl determinations. In order to obtain a sharp endpoint, the distillate before titration was reduced in volume to 50 cc. by distillation under vacuum. The mean deviation of 42 determinations approximated 0.002 mgs. of nitrogen per gram of sample.

The adsorbed nitrogenous material was assumed to consist entirely of lactalbumin, and calculations of the weight of adsorbed material were made on this basis. The total area of the films was calculated upon the assumption that the individual bubbles were spherical. However, the shape was more nearly that of a distorted ellipsoid, and consequently the values calculated for the quantity of adsorbed material should be considered higher than those corresponding to the actual shape. For comparative purposes such as these experiments are intended, absolute adsorption values are of no particular significance. It is only necessary that the bubbles in the various solutions should be uniform in size inasmuch as Gans and Harkins (9) have shown that the degree of adsorption varies with the size of the bubble.

Results

The results given in table 3 indicate that over a wide range of dilutions, the adsorption values of milk serum are nearly constant, and hence that the adsorption film may be considered as saturated even in the most dilute

TABLE 3

The degree of adsorption of protein from milk serum and from milk serum containing 1 per cent by volume of skim milk

SOLUTION	MILK SERUM			MILK SERUM + 1% SKIM MILK		
	1	2	3	1	2	3
	Mean of 2 expts.	Mean of 4 expts.		Mean of 2 expts.	Mean of 3 expts.	
Dilution—						
pts. H_2O per 100 pts. serum	0.00	66.7	900	0.00	66.7	100
Conc. N. original sol. mgs.	0.580	0.437	0.0910	0.702	0.519	0.394
Conc. N. residual sol. gm.	0.568	0.422	0.0822	0.688	0.500	0.386
Conc. N. foam gm.	0.601	0.458	0.1132	0.724	0.543	0.410
Wt. of foam gm.	63.88	55.92	70.91	66.71	52.49	74.71
Area of bubble cm^2	3.38	3.87	3.80	3.42	3.65	3.37
Total area $cm.^2$	61,330	61,380	80,210	63,200	70,400	58,600
Adsorption of protein gms./$cm.^2$	1.8×10^{-7}	1.7×10^{-7}	1.5×10^{-7}	1.9×10^{-7}	1.6×10^{-7}	1.6×10^{-7}

serum solution. Upon the addition of 1 per cent by volume of skim milk the surface remains saturated with respect to the quantity of adsorbed nitrogenous materials; and consequently the decrease in the foaming power of the serum cannot be explained on the basis of a change in the closeness of packing of the adsorbed molecules, in substantiation of the results obtained by means of surface tension measurements, and decidedly in conflict with the views expressed by Eichholtz.

Spreading Power and Foaming

The change in the foaming power of the serum may be demonstrated to be due to the introduction of milk fat. Concomitantly forces evidently originate tending toward the destruction of a liquid film. The theories advanced by Van Dam and Sommer and Horral, as described in the introduction, are concerned with these destructive forces. That proposed by Van Dam leads to the conclusion that the destructive action should be independent of the spreading power of the emulsified fat or oil; that proposed by Sommer and Horral suggests that the less the tendency of the fatty material to spread the greater its tendency to destroy the film. Both theories lead their respective authors to the conclusion that the coarser the dispersion formed from the lipoid substances the greater the destructive action of this material. That these theories are open to criticism may be demonstrated by a simple experiment. Thus, Squibbs mineral oil, which does not spread on water and which forms a coarse dispersion with milk serum, exerts only an insignificant depressant action on the foaming power of the serum in contradistinction to the marked depressant action of butterfat, oleic acid, or triolein, substances which spread on water and which form fine dispersions with milk serum.

Edser (10) has pointed out the resemblance existing between the spreading of a rupture in a soap bubble and the spreading of a film of grease over pure water. The contraction of the soap bubble due to the spreading of a rupture has its analogue in the contraction of the surface of pure water due to the spreading of a film of grease. This analogy suggests that the presence in a thin liquid film of a substance which will spread may be considered tantamount to the existence of a rupture with the obvious exception that in the case of the spreading substance a thin film of the substance would remain, the stability of which would be such that it would also vanish quite readily. Where the size of the globule of spreading substance is insufficient to displace the entire film, a region of weakness would exist in that portion of the film displaced by the spreading substance.

The rupture of a highly viscous lamella of ice cream mix may be observed to spread quite slowly. If a drop of linseed oil is placed on such a lamella the spreading of the oil occurs at a slow rate, and the displacement

of the entire lamella by the oil may be observed. In this manner the comparatively stable ice cream mix lamella is replaced entirely by an unstable oil lamella.

It is proposed that the observed decrease in foaming power occurring when skim milk is added to milk serum is capable of interpretation on the basis of the equivalence noted between a rupture and the presence of a spreading substance in a thin film. The contractile forces arising with the formation of a liquid film, as a result of the tendency of the fat globules to spread, effect the destruction of the film in much the same way as a mechanical rupture.

It is interesting to note that the foaming power of milk decreases with an increase in temperature from 80° C. to 20° C. King (11) has recently presented evidence to show that the portion of the surface of milk covered with a film of fat increases with a rise in temperature from 10° C. to 20° C. These results, therefore, indicate that the decrease in foaming power may be explained on the basis of the increase in the tendency of the fat to spread as the temperature rises.

SUMMARY

The action of the milk fat in milk as a foam depressant has been discussed in connection with the theories which have been proposed to explain the phenomenon. The consequences of these theories have been investigated experimentally. Contrary to some of the ideas expressed adsorption experiments indicate that the action of milk fat is not due to the removal by the fat of the protein adsorbed at the air/milk serum interface; and surface tension, and superficial viscosity measurements indicate that the action is not attended by any significant changes in the dynamic and static surface tension, or in the superficial viscosity of milk serum.

The destructive action of milk fat and other lipoids on foam depends in some way upon the ability of these substances to spread on pure water, and consequently the theories relating to the destructive action of fat on foam which do not take into consideration the importance of spreading, are open to criticism.

It has been pointed out that the presence in a thin film of a globule of a substance which will spread may be considered tantamount to the existence of a rupture, and the suggestion has been made that the decrease in foaming power occurring when skim milk is added to milk serum is capable of interpretation on the basis of the equivalence noted between the existence of a rupture, and the presence of a spreading substance in a thin film.

Procedures have been described for the measurement of dynamic surface tension, and of an index of superficial viscosity.

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THE DISTRIBUTION OF PHOSPHOLIPOIDS IN CREAM

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The variation of phospholipoids with the fat contents of creams, their probable relationship to the colloidal systems of milk products and their concentration to effect the presence of maximum quantities of a certain definite form of assimilable phosphorus were the chief considerations leading to this investigation.

In spite of the accepted recognition of the excellent emulsifying and other properties of lecithin and its allied phospholipoids rather little is found in the literature relative to their physical-chemical functions in milk and cream systems. Palmer and Wiese (1) (2) recently, in studying substances adsorbed on the fat globules in cream, substantiated evidence indicating that phospholipoids together with a mixture of certain proteins are invariably present as a stabilizing mixture in conjunction with the fat globule, and found that artificial milks prepared by dispersing milk fat in an aqueous suspension of (egg-yolk) phospholipoid yielded cream which separated more nearly like natural cow's milk than like milk fat dispersions having buttermilk, calicum caseinate, lactalbumin, and globulin respectively in the stabilizing sols. This observation indicates the possible importance of the presence of lecithin in milk and cream with regard to its serving to assist in the maintenance of the colloidal system.

The results of previous investigators indicate that the distribution of phospholipoids in milk products varies rather widely. The amount present in milk is reported to vary from 0.040 to 0.117 per cent. Bordas and de Raczkowski (3) found 69 per cent of the total phospholipoids passing into the cream upon separation. Gilkin (4) substantiates this and shows that about 3 per cent is retained by the skim-milk fraction while Dornic and Daire (5) report larger amounts to be retained in the skim milk. The separator slime (6) was found to comprise an appreciable amount of phospholipoids—about 12.5 per cent. When cream is churned phospholipoids are removed and the resultant butter contains lesser quantities than does the original cream (2) (7) (8) (9). These quantities, however, are appreciable and not mere traces as reported by some authors. Cusick, whose results are cited by Supplee (9), reports 0.0723 per cent di-stearyl lecithin for raw sweet butter, but when his values are recomputed on the basis of his recovered lipoid phosphorus they are found to be actually twice the reported amounts or approximately 0.14 per cent. Smith's value for lipoid phosphorus in sweet butter (10) is found to be equivalent also to 0.14 per cent of di-stearyl lecithin, thus checking Cusick's corrected value.

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Wiese, Nair and Fleming (11) maintain that the phospholipoid content of cream is very uniform and, working with 40 per cent cream, found it to be equivalent to 8 mg. of phosphorus per 100 grams for summer cream and 7.2 mg. per 100 grams of winter cream; and, computing their values on the basis of total fatty extract, report 19.9 and 18.2 mg. per 100 grams, respectively. Their statement is based on results obtained with cream of a definite unvarying fat content and probably produced under the same uniform conditions and, as will be demonstrated later in this work, cannot be applied to cream in general.

Observations made in this laboratory during the preliminary examination of creams of varying fat concentrations for their lipoid phosphorus content, revealed that a more or less definite relationship seemed to exist between the two components. In general, it appeared that the phospholipoid content increased almost uniformly with the fat content until a certain point of fat concentration was reached when it began to diminish appreciably. On the basis of the fatty extract there was an obvious decrease in lipoid phosphorus with the increase of the fat content of the cream. In table 1 are shown the lipoid phosphorus and fat values obtained during the preliminary examination of pasteurized market milk and cream.

TABLE 1

Fat and lipoid phosphorus contents of market creams

SAMPLE	FAT	LIPOID PHOSPHORUS	LIPOID PHOSPHORUS IN FATTY EXTRACT
	per cent	*mg. per 100 grams*	*mg. per 100 grams*
1*	4.02	1.14	28.4
2	16.33	3.1	19.0
3	32.68	5.4	16.6
4	34.90	5.7	16.3
5	35.58	6.2	17.5
6	41.24	6.0	14.5
7	43.49	5.7	13.1
8	47.30	5.6	10.9

* Milk.

These few preliminary values present the possibilities of an interesting picture. The phospholipoid increase on the basis of the cream as such with the increase in fat and the decrease on the basis of the fatty extract established the fact that a certain loss of phospholipoid takes place as creams of increasing fat contents are produced. In short, a great portion of the phospholipoid content is removed with the skim milk.

EXTRACTION METHODS

It has been found that in the fatty residues obtained by the Mojonnier modification of the Roese-Gottlieb procedure for the determination of fat in

milk products are included also the milk phospholipoids. For this reason it has been recommended that these residues be employed for the determination of lipoid phosphorus (10), (12), (13). Wiese, Nair and Fleming (11) with this method were able to recover quantitatively known amounts of lecithin in simple aqueous dispersions and also in the presence of milk fat. Chapman (12) experienced difficulty in the recovery of added amounts of lecithin in butterfat. The writer (14) found that slightly higher results could be obtained by employing a mixture of absolute alcohol and chloroform as the initial solvent but a persistent flourescence carried through to the final solutions of the fatty extracts indicated possible contamination with phosphorus in other forms of combination and the method was temporarily discarded.

Holm, Wright and Deysher (19), using a method dependent upon the solvent action of a mixture of 3 parts of 95 per cent alcohol and 1 part of ethyl ether with no provision for subsequent purification of their extracts to remove phosphorus from inorganic and other sources secured results indicating the presence of phospholipoids to the extent of from 0.152 to 0.158 per cent in milks of from 2.5 to 4.3 per cent fat content. In creams having from 27 to 42 per cent fat content they report phospholipoid values of from 0.224 to 0.284 per cent. In the entire range of milk and cream of from 2.5 to 42 per cent fat contents their reported values increase from 0.152 to 0.284 per cent while the writer, working with purified extracts secured results of from 0.03 to 0.17 per cent from the same range of fat contents. The values obtained with the purification step increase from approximately 20 per cent to 61 per cent of the values reported by Holm *et al.*, thus approaching agreement with increasing fat content and attendant decreasing moisture. Obviously the presence of small quantities of moisture in the Holm extraction procedure from the source of the alcohol as well as from the milk or cream itself allows the solution of considerable phosphorus other than that of the phospholipoids. Extracts made by the writer when applying this procedure to milk continued depositing solid material despite repeated filtration for about 24 hours losing up to 17 per cent of their original phosphorus. One extract yielding 0.173 per cent of phospholipoids upon immediate analysis filtered 24 hours later gave only 0.144 per cent. Another sample of the same milk to which was added 0.3 per cent of the mixed inorganic phosphates normally present in milk yielded 0.280 per cent of phospholipoids and when filtered 24 hours later only 0.209 per cent. Still another sample of milk showing 0.028 per cent of phospholipoids by the Mojonnier modification of the Roese-Gottlieb extraction, drying and re-extraction with chloroform, gave 0.178 per cent by the Holm procedure. Addition of 0.1 per cent of KH_2PO_4 increased the latter value to 0.199 per cent and 0.065 per cent K_2HPO_4 added to another portion increased it to 0.196 per cent while the original value of 0.028 per cent remained unaltered

by these additions. The moisture present in the extracting mixture of the Holm procedure evidently carries with it inorganic phosphorus and thus yields high phospholipoid values.

It was finally decided most convenient and satisfactory to employ the Mojonnier modification of the ammoniacal Roese-Gottlieb procedure for the extraction of the total fat and lipoids. Three extractions were made using a ten-gram sample and the combined solvent was evaporated to dryness on a steam bath in a tared 150 cc. fat flask with the aid of a gentle current of air from an electric fan. The fatty extract was then dried in an oven at 100° C. to constant weight (60 to 90 minutes) and the percentage of total fat calculated.

COLORIMETRIC DETERMINATION OF LIPOID PHOSPHORUS

The fatty residue as obtained above was dissolved in a small amount of chloroform and transferred by means of small portions of the solvent from a wash bottle to a 50 cc. volumetric flask through a small funnel containing a closely packed extracted pledget of cotton. The volume was then adjusted to the mark and suitable aliquots were removed for the determination of the phosphorus.

An aliquot containing about 0.10 mg. of phosphorus was transferred to a small flat-bottomed platinum dish and evaporated to dryness on the steam bath together with 1 cc. of a 50 per cent alcoholic solution of magnesium nitrate with the aid of a gentle current of air from an electric fan. The dish with the residue was then placed on a hot plate and the heat gradually increased until a dry charred mass resulted. The char was finally ignited to a white ash in a muffle at about 500° C. The phosphorus was determined in the residue according to Smith's modification of the Bell-Doisy procedure for blood phosphorus (10). After moistening with a few drops of distilled water 2 cc. of diluted sulphuric acid (1+1) were added and complete solution was effected. The perfectly clear and colorless resulting solution was then carefully transferred to a 200 mm. test tube calibrated with a mark at 20 cc., the dish washed several times with distilled water, the washings being added to the tube, and the volume finally adjusted to the mark. A standard was prepared in an identical tube by treating 2 cc. of a standard potassium dihydrogen phosphate solution (0.2193 gm. per litre = 0.05 mg. per cc.) (15) with the acid and making up to volume. The reagents were then added as follows: 2 cc. of 15 per cent sodium bisulphite containing 0.5 per cent. hydroquinone, followed by 2 cc. of ammonium molybdate according to Briggs (15) – 25 gm. ammonium molybdate C.P. in 300 cc. H_2O plus 200 cc. H_2SO_4 (3+5). The contents of the tubes were then mixed well, immersed in a boiling water bath for 15 minutes, cooled in running water and made up to 50 cc. volume prior to comparison of the clear blue color in a colorimeter.

PHOSPHOLIPOID-FAT RELATIONSHIPS IN CREAM

Seven samples of cream of increasing fat content were prepared in the laboratory from the same batch of fresh raw milk by simple centrifugalization in an International type SB centrifuge and analyzed for their total fat and lipoid phosphorus contents by the methods outlined. The results are shown in table 2.

TABLE 2

Fat and lipoid phosphorus contents of creams made by simple centrifugalization of the same raw milk

CREAM FRACTION	FAT	LIPOID PHOSPHORUS	LIPOID PHOSPHORUS IN EXTRACT
	per cent	*mg. per 100 gms.*	*mg. per 100 gms.*
Raw Milk	3.52	1.13	32.10
1	4.47	1.25	27.96
2	11.16	2.37	21.24
3	19.95	3.53	17.69
4	34.58	5.57	16.10
5	36.52	5.78	15.83
6	40.26	6.16	15.30
7	68.87	6.18	9.97

The values obtained on these samples in general checked those obtained in the preliminary work on the market creams (table 1) to the point of approximately 35 per cent fat content. Above this point, however, the lipoid phosphorus content continued to rise with the fat content and the actual point of transition at which the phospholipoids began to diminish as the fat content continued to rise occurred at an obviously higher fat concentration. This might indicate that phospholipoids are destroyed during the pasteurization or aging of cream prior to marketing. Further consideration is given this possibility later in this work and in a subsequent paper.

The wide difference of fat content between samples 6 and 7 rather obscured the actual point of transition at which the phospholipoids begin to decrease as the fat increases continue. It was therefore decided to prepare a set of samples of cream of increasing fat contents according to strict modern dairy practice. Accordingly, sixteen such samples were made in a standard cream separator of the skim-milk screw type using the same batch of fresh raw milk. The fat content was increased by careful regulation of the skim-milk screw. The samples were quickly cooled immediately after their separation and kept at approximately 40° C. until analyzed. The fat and lipoid phosphorus contents were then determined in the raw samples and also after pasteurization at 143° F. for 30 minutes. The results are given in table 3.

It is noted that the values indicating the rise in phospholipoid content as the fat content increases check those given in table 2 rather closely and

TABLE 3

Fat and lipoid phosphorus contents of creams prepared in separator from same fresh raw milk before and after pasteurization

CREAM FRACTION	FAT		LIPOID PHOSPHORUS		LIPOID PHOSPHORUS IN EXTRACT	
	Before pasteurization	After pasteurization	Before pasteurization	After pasteurization	Before pasteurization	After pasteurization
	per cent		*mg. per 100 grams*		*mg. per 100 grams*	
Milk	3.12	3.07	0.91	0.94	29.17	30.62
Cream 1	10.46	10.39	1.97	2.10	18.83	20.21
" 2	11.48	11.51	2.25	2.36	19.60	20.50
" 3	13.34	13.39	2.44	2.51	18.30	18.74
" 4	16.00	15.99	2.93	3.01	18.31	18.82
" 5	17.11	17.10	3.22	3.22	18.82	18.83
" 6	19.20	19.15	3.41	3.49	17.76	17.90
" 7	21.39	21.39	3.89	3.61	18.18	16.88
" 8	25.36	25.30	4.33	4.34	17.07	17.15
" 9	37.68	37.81	6.32	6.15	16.77	16.27
" 10	46.77	46.85	7.59	7.78	16.23	16.60
" 11	55.24	55.38	8.49	8.71	15.37	16.72
" 12	58.37	58.48	8.33	8.42	14.27	14.40
" 13	58.69	58.87	8.26	8.45	14.07	14.36
" 14	59.12	59.17	8.14	8.69	13.77	14.69
" 15	59.09	59.15	8.16	8.59	13.80	14.52
" 16		66.63		8.13		12.20

those in table 1 for the creams having fat contents up to 35 per cent. The results shown in tables 2 and 3 are plotted graphically in figures 1 and 2 for the purpose of comparison and visualization of what has occurred.

The transition point (figs. 1 and 2) occurs at 55 to 58 per cent fat content. This seems to be evidence to indicate that rapid coalescence or aggregation of the fat globules has taken place in the cream system at this fat concentration and that there is about to take place a partial reversion of the colloidal system. This can be better understood when it is remembered that lecithin possesses the property of swelling up considerably in aqueous medium and, therefore in milk and cream it probably occupies all of the space between the fat globules forming an aqueous phospholipoid "matrix" for their dispersion. It can accordingly be conceived that the volume of this phospholopoid "matrix" which is distributed uniformly throughout the continuous phase is diminished with the removal of the skim milk, the phospholipoid being itself removed in part.

In figure 2 we note that about 40 per cent of the phospholipoid is removed from the fatty extract as the fat content of the cream reaches approximately 15 to 20 per cent. Then, until the concentration of the fat approaches about 55 per cent, the phospholipoid tends to adhere rather tenaciously to the fat globule. For this reason it is noted that the lipoid phos-

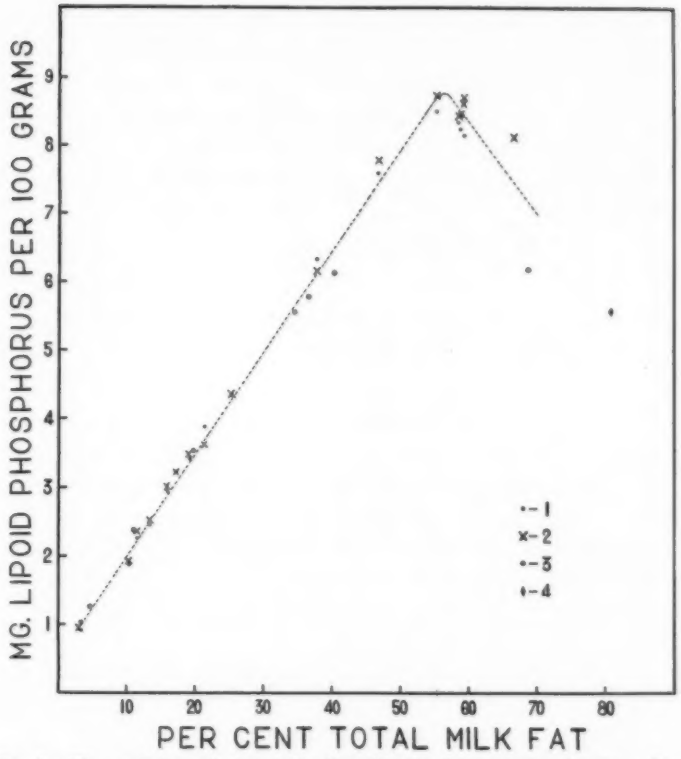

FIG. 1. The variation of phospholipoids with the fat contents of cream: 1—cream produced in cream separator from same batch of raw milk; 2—same cream after pasteurization; 3—cream produced in laboratory by ordinary centrifugalization of same batch of raw milk; 4—butter (Cusick; Smith).

phorus content of the fatty extract remains rather constant in this range, the milk serum carried out with the skim milk taking with it a uniform quantity of the phospholipoid. This obviously concentrates the remaining phospholipoid about the fat globule and results in the formation of a fragile membrane around it, and as more serum is removed the fragility of this membrane is increased until it finally gives way and allows fusion of the unprotected fat mass. The fat becomes continuous and probably occludes a portion of globules surrounded by unbroken membranes. King (16) in a recent work explains butter as a system containing fat in both the continuous and dispersed phases. He maintains that it comprises globules of water and globules of fat surrounded with a stabilizing film in a fatty liquid. This accounts for the presence of phospholipoids in butter. They

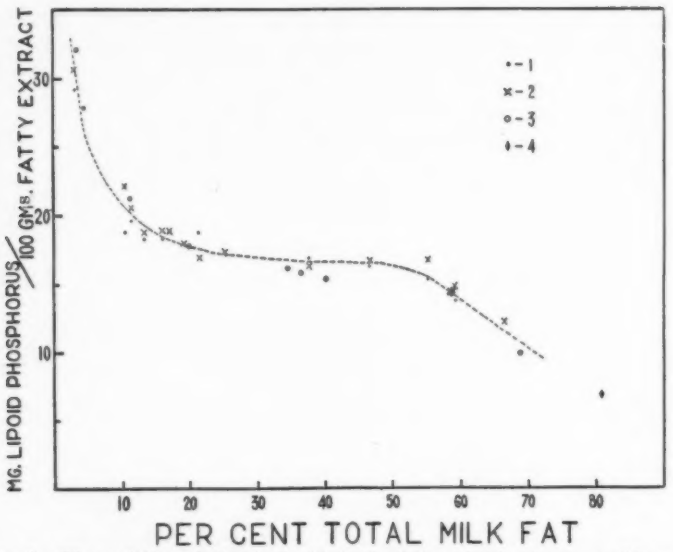

FIG. 2. The variation of phospholipoids in the fatty extracts of creams of increasing fat contents: 1—cream produced in cream separator from same batch of raw milk; 2—same cream after pasteurization; 3—cream produced in laboratory by ordinary centrifugalization of same batch of raw milk; 4—butter (Cusick; Smith).

remain due to mechanical occlusion during churning rather than to solution in the fat as is demonstrated by the fact that filtered melted butterfat contains no phosphorus.

It is interesting to note (figures 1 and 2) that Cusick's and Smith's figures for lipoid phosphorus in butter fall directly on the line of phospholipoid decrease, thus substantiating the facts discussed.[1]

To demonstrate that the original milk phospholipoids are divided between the cream and the skim milk prepared from it, samples were taken from a separator run at a local dairy and analyzed as follows:

100 GRAMS MILK—4.24 PER CENT FAT
containing 1.06 mgs. lipoid phosphorus

9.8 grams cream – 41.53 per cent fat contains 5.86 mgs./100 gms. lipoid P or .57 mg.	90.2 grams skim – 0.08 per cent fat contains 0.53 mg./100 grams lipoid P or 0.48 mg.

loss – 0.01 mg. lipoid P

[1] Cusick's figure (9) for lipoid P_2O_5 in butter is equivalent to 5.56 mg. of phosphorus per 100 grams and Smith (10) gives as averages for 7 samples of sweet butter 5.58 mg. of phosphorus per 100 grams and 80.85 per cent of butter fat.

Another set of samples prepared from raw milk in the laboratory showed the following distribution:

100 GRAMS MILK – 5.35 PER CENT FAT
containing 1.32 mgs. lipoid phosphorus

9.9 grams cream – 37.22 per cent fat contains 5.44 mgs./100 gms. lipoid P or 0.54 mg.	90.1 grams skim – 0.168 per cent fat contains 0.82 mg./100 gms. lipoid P or 0.74 mg.

loss – 0.04 mg. lipoid P

The distribution of lipoid phosphorus in creams of increasing fat contents and in the resulting skim milk prepared from 100 gram portions of milk is shown in figure 3.

EFFECTS OF PASTEURIZATION

An attempt was made to verify information given in the literature to the effect that heat causes a diminution in milk phospholipoids (18). The raw milk and cream samples, the results for which are given in table 3 and plotted in figures 1 and 2, were subjected to a pasteurization temperature of 61–62° C. for 30–35 minutes, cooled, and analyzed for fat and lipoid phosphorus contents as before. No decrease in phospholipoids was effected by the heating procedure as is evident by the results given in the table and graphs. Further work on the effect of heat on milk phospholipoids is reported in a subsequent paper (17).

CONCLUSIONS

(1) The phospholipoid content of fresh cream increases uniformly with the fat content to a point of approximately 55–58 per cent milk fat when it diminishes with further fat increases.

(2) The phospholipoid content of the fatty extract of fresh cream shows a variable decrease with the increase of the fat content of the cream indicating a variable loss of the original milk phospholipoids.

(3) Approximately 40 per cent of the original milk phospholipoids are removed with the skim-milk in producing cream of from 15 to 20 per cent fat content.

(4) Little further removal of phospholipoids occurs as creams of from 20 to 55 per cent fat content are produced.

(5) Evidence presented indicates the probable occurrence of a reversion of the colloidal system in fresh cream at approximately 55–58 per cent fat content.

(6) No indications were found that heat alone will effect the destruction of phospholipoids in milk products. Further work on the effect of heat on milk phospholipoids is reported in a subsequent paper.

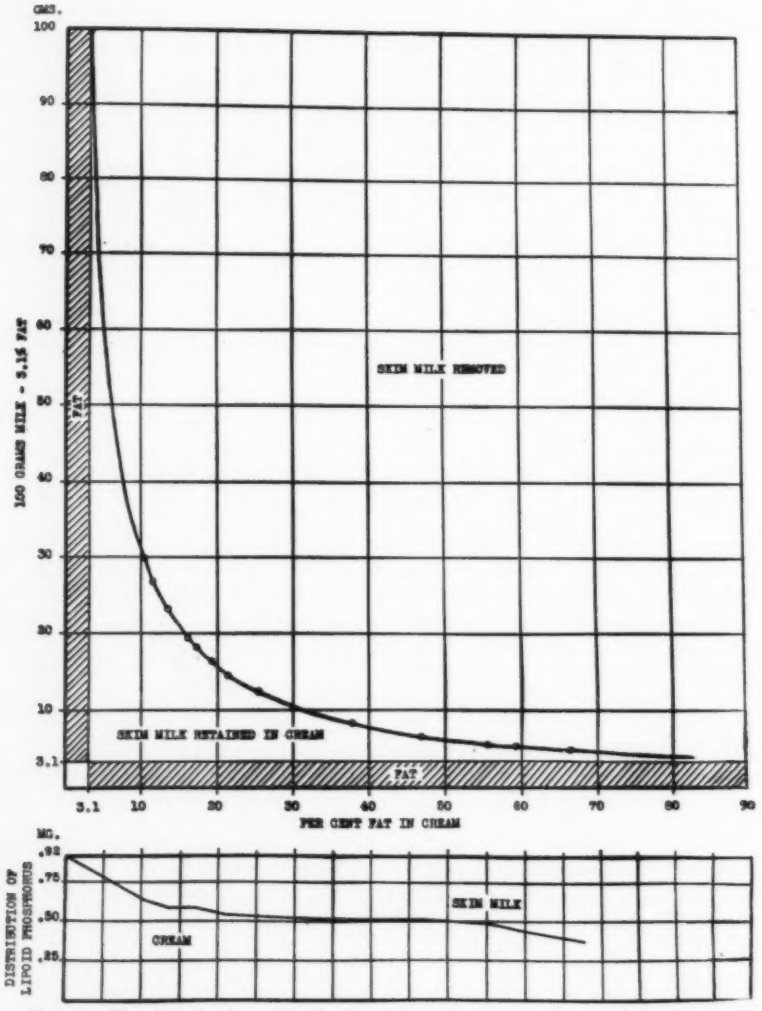

FIG. 3. The distribution of milk lipoid phosphorus in cream and resultant skim milk as creams of increasing fat content are produced from 100-gram portions of milk. (Based on table 3.)

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THE EFFECT OF HEAT ON MILK PHOSPHOLIPOIDS

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The destruction of phospholipoids in milk and milk products during heating is by many considered an accepted and unchallenged truth. Bordas and de Raczkowski (1) in 1903 conducted heating tests on milk and concluded that a considerable portion of the total lecithin present was destroyed at elevated temperatures maintained during 30 minute periods of time. Thus they reported that a sample heated for 30 minutes at 60° C., simulating ordinary pasteurization, lost 14 per cent of its original lecithin content. At 80° C. this was doubled, while no greater losses occurred at 95° C. Another sample heated at 95° C. lost but 12 per cent of its phospholipoid content computed as lecithin while a third sample when held in an autoclave at 105–110° C. for 30 minutes lost 30 per cent of its original lecithin content. It is obvious that no uniform relationship between heating and phospholipoid destruction exists in these reported figures contrary to expectations that such decomposition, if due entirely to heat, would be directly proportional to the degree of heat as well as to the heating time. The results of Bordas and de Raczkowski's work are given in table 1.

TABLE 1

The destruction of milk phospholipoids by heating over thirty minute periods—Bordas and de Raczkowski

LECITHIN CONTENT	HEATING TEMPERATURE	LOSS
per cent	*Heating temperature*	*per cent*
0.0252	60	14
0.0252	80	28
0.0252	95	28
0.0365	95	12
0.0255	105–110	30

In attempted verification of the above, 16 samples of cream of increasing fat content separated from the same batch of fresh raw milk were analyzed for their lipoid phosphorus contents, subjected to a pasteurization temperature of 61–62° C. for 30–50 minutes, cooled, and again analyzed as before (2). A drop of diluted formaldehyde had been added to the raw samples for the purpose of retarding bacterial growth subsequent to the removal of portions for the initial analyses. No decrease in phospholipoids was effected by the heating procedure (see table 3 in preceding paper).

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At this point it is interesting to consider Cusick's recomputed values for the lecithin contents of various butters (3). Raw sweet butter showed a decrease in lecithin of from 0.145 per cent to 0.104 per cent in 48 days and no further decrease to 72 days. Pasteurized sweet butter decreased in lecithin content from 0.145 per cent to only 0.136 per cent in 48 days and to 0.101 per cent in 72 days. Raw ripened butter showed a lecithin decrease of from 0.139 per cent to 0.098 per cent in 48 days and to 0.094 per cent in 72 days while pasteurized ripened butter having a lecithin content of 0.090 per cent showed no decrease in 72 days. No initial decrease is shown for sweet butter upon pasteurization while raw ripened butter has 4 per cent less lecithin and pasteurized ripened butter 40 per cent less lecithin. These figures indicate unmistakable bacterial enzymatic decomposition of the lecithin. Apparently the lecithin decomposing enzyme produced during the bacterial ripening process has a rather high optimum temperature as is indicated by the 40 per cent lecithin loss in the pasteurized ripened butter as compared to only a 4 per cent loss in the raw ripened butter.

The possibilities of bacterial enzymatic decomposition of phospholipoids have been studied previously by the writer (4). It was found that there exist certain bacteria which produce powerful extracellular lecithin-splitting enzymes and several strains were successfully isolated from chance inoculated whole mixed eggs. It is probable that bacterial phospholipoid-splitting enzymes having a possible high optimum temperature played a significant rôle in yielding the previously cited results reported by Bordas and de Raczkowski as well as those of Cusick.

To secure further evidence that heat alone is not the factor responsible for the destruction of milk phospholipoids a sample of fresh raw milk produced under very carefully controlled sanitary conditions and having an initial bacterial plate count of 3,500 per cc. was obtained, analyzed for lipoid phosphorus content, and 100 cc. portions subjected to the action of heat and known phospholipoid-splitting bacteria. The portions were then again analyzed to ascertain any loss in phospholipoid content. The treatments and results obtained are given in table 2.

It is evident that heat causes no change in the phospholipoid content of the milk. Higher autoclaving temperatures than those used by the previous investigators were applied to portions 3 and 4. Very evident caramelization occurred in portion 4. The total time this portion remained at a temperature above 100° C. was well over an hour, yet no diminution of phospholipoids occurred. The slight differences in the results can be regarded to be well within experimental error.

It might be well to mention at this point that continued heating at 100° C. of the extracted fatty residues of both egg and milk products after constant weight is obtained yields no diminution in lipoid phosphorus.

The bacteria present in the milk upon its receipt were obviously not of the lecithin-splitting type. This is doubtlessly due to the carefully con-

TABLE 2

The effect of heat and lecithin-splitting bacteria on milk phospholipoids.

PORTION AND TREATMENT	TOTAL FAT	LIPOID PHOSPHORUS	LIPOID PHOSPHORUS IN EXTRACT
	per cent	*mg./100 grams*	*mg./100 grams*
1. Raw fresh milk as received	3.98	1.20	30.15
2. Pasteurized portion—kept at 60–65° C. for 30 minutes	4.05	1.18	29.14
3. Autoclaved portion—kept at 120–125° C. for 15 minutes	4.07	1.22	30.00
4. Autoclaved portion—kept at 120–125° C. for 30 minutes	4.03	1.23	30.52
5. Raw portion—incubated at 37°C. for 48 hours	3.90	1.21	31.02
6. Raw milk plus 1% 48 hr. broth suspension of lecithin-splitting bacteria incubated at 37° C. for 24 hours	3.99	0.54	13.53
7. Portion No. 6—incubated at 37° C. for 48 hours	3.98	0.43	10.81

trolled sanitary conditions under which the milk was produced. A gassy curd was produced after 48 hours at 37° C. but there was no decrease in the phospholipoid content.

Portion 6 and 7 were inoculated with a 48 hour broth culture of the tentatively named *Bacillus lecitosis, nov. sp.* which had been isolated from decomposing mixed eggs into which it found its way by chance inoculation. This microorganism has been found to produce an extra-cellular enzyme capable of destroying completely the phospholipoids present in eggs (4). In milk it was found to destroy 65 per cent of the phospholipoids in 48 hours at 37° C. At least three other organisms capable of producing lecithin-decomposition have been isolated in this laboratory but their identification is as yet incomplete.

The experimental technic and methods employed in this work were identical to those used in the previously reported study of the phospholipoid distribution in cream (2).

SUMMARY

No evidence that heat alone causes the destruction of lecithin and its allied phospholipoids in dairy products was obtained. This is in contradiction to previously reported results obtained without the consideration of the

probable presence of phospholipoid decomposing bacterial enzymes. It is shown that certain bacteria are capable of producing enzymes which will effect the destruction of milk phospholipoids.

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A WATERING DEVICE FOR EXPERIMENTAL WORK

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Some types of experimental work with dairy cattle require that the water intake be measured. When adequate numbers of experimental animals are used, the cost of water meters to individual stalls is often prohibitive. The accompanying cut shows our solution of this problem.

A used ten gallon milk can was mounted on a plank frame which was high enough to allow attachment of a standard Star drinking cup. The cup was attached to the can by means of standard pipe and fittings. The attachment to the can was by means of lock nuts and a rubber gasket.

Water was weighed or measured into the can and unused portions weighed back or the amount required to refill the can was recorded. Very little difficulty was experienced with leakage or spillage, and the device, although inexpensive, was altogether satisfactory for our purpose.

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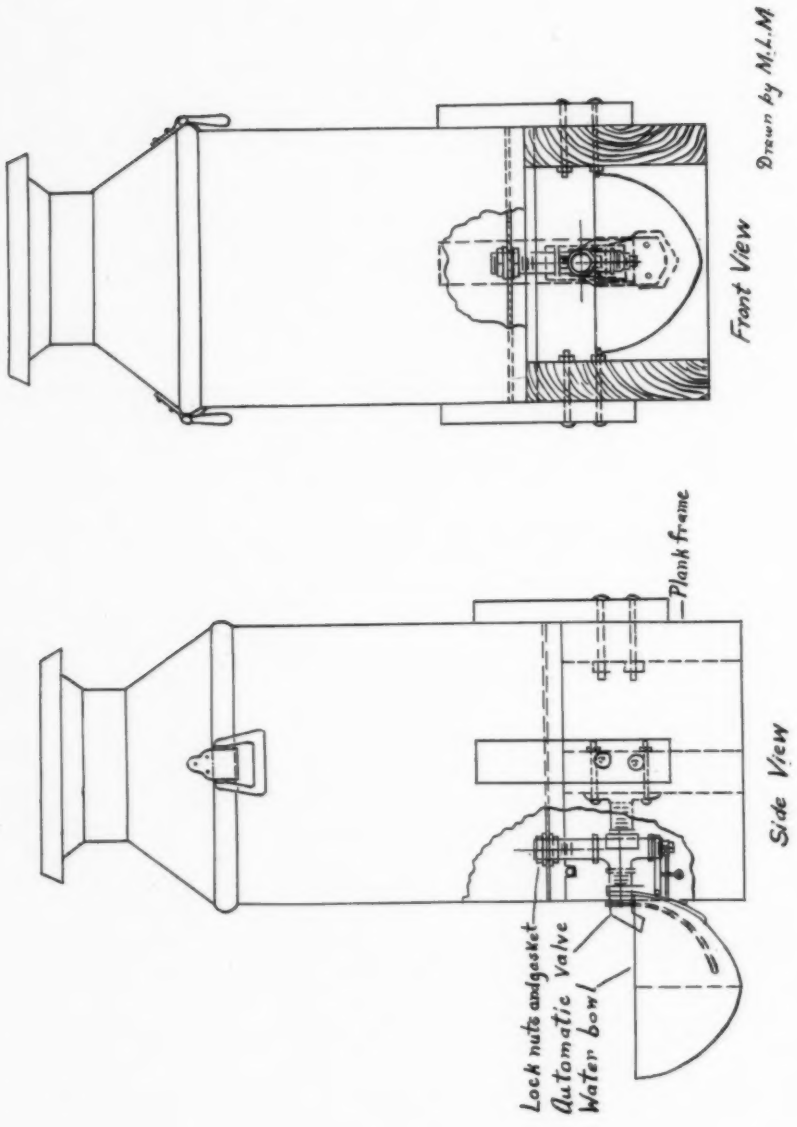
Front View
Drawn by M.L.M
Side View
Plank frame
Lock nuts and gasket
Automatic Valve
Water bowl

A STUDY OF THE RELATION OF MATERIALS ADSORBED ON THE FAT GLOBULES TO THE RICHNESS OF FLAVOR OF MILK AND CERTAIN MILK PRODUCTS*

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The peculiarly rich flavor of buttermilk was noted by Sammis (8) in 1914 and again (9) in 1920. The authors of this paper have observed repeatedly that the buttermilk resulting from the churning of sweet cream possesses a flavor richness that is about equivalent to that of normal whole milk, although the butterfat content of whole milk usually is about eight times that of buttermilk. It seemed likely, inasmuch as buttermilk is relatively richer in phospholipids than whole milk or skimmilk, that these compounds might be responsible in some way for a large portion of the flavor richness observed in buttermilk. It also seemed obvious that, if phospholipids contribute to the rich flavor of buttermilk, they also should be responsible for a portion of the richness of flavor in milk products which contain them, whereas milk products so treated as to remove a portion of the phospholipids might be expected to have lost some of their flavor richness.

An excellent summary of the findings of various workers relative to the phospholipid content of various milk products is given by Holm, Wright and Deysher (5). These authors also have determined the phospholipid content of milk and the products manufactured from it. Their work indicates that the greater portion of the phospholipids of normal milk occurs in the plasma and not adsorbed on the fat globules, as over 70 per cent of the phospholipids remained in the skimmilk after separation. However, their results confirmed the suggestion of Thurston and Petersen (11) that the phospholipid content of buttermilk increases as the butterfat content of the cream churned increases. This is shown by the excerpts from their data given in table 1.

Palmer and Samuelson (6) and Palmer and Wiese (7) have shown that phospholipids form a part of the adsorbed film on the fat globules in normal milk, and that these substances are recovered in large proportions in the buttermilk after churning cream washed eight times by dilution with water and reseparation. These findings explain why the phospholipid content of buttermilk is higher than that of whole or skimmilk and indicate that an increase in phospholipid content of buttermilk should be expected when an

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** Most of the data presented herein were included in a thesis presented by J. L. Barnhart in partial fulfillment of the requirements for the degree of Master of Science, West Virginia University.

TABLE 1

The phospholipid content of buttermilk from cream of varying richness (After Holm, Wright and Deysher (5))

FROM MILK NO.	BUTTERFAT CONTENT OF CREAM	PHOSPHOLIPID CONTENT OF BUTTERMILK
	Per cent	*Per cent*
1	27.0	0.284
2	34.0	0.318
3	42.0	0.382

increase in the butterfat content of the cream from which it is churned occurs because of the concentration of an increased amount of "hulls" in a reduced quantity of plasma.

At the West Virginia Station a method has been used of estimating the phospholipid content of dairy products based on extraction of the Roese-Gottlieb fatty residue with three ten-ml. portions of acetone, evaporating the acetone, weighing the residue considered to be butterfat, and determining the phospholipids by difference. This method admittedly yields only approximations, as it has been shown (1) (2) (5) that ether extraction as carried out in the Roese-Gottlieb procedure fails to remove all the phospholipids from the plasma. However, when applied to buttermilk, this method yields differences which are in line with the theory, and has been shown (10) to be very accurate for prepared fat-phospholipid mixtures. Table 2 shows these results. The data indicate, as do those of Holm *et al.* (5), that the phospholipid content of buttermilk increases as the fat content of the cream churned increases.

In view of the foregoing information the production of buttermilk from very rich cream was selected as a means of obtaining a milk product relatively rich in phospholipids. For purposes of flavor comparisons, milk products of medium and low phospholipid content were desired also. Whole milk standardized with skimmilk to the percentage butterfat content of the buttermilk served as the product of medium phospholipid content. The preparation of a milk product of low phospholipid content was accomplished by emulsifying washed butterfat in skimmilk by means of a homogenizer at pressures such that the fat globules were approximately of the same average size as those in the original whole milk as determined by comparison under the microscope.

The washed butterfat was prepared by repeatedly diluting cream and separating it in a de Lavel No. 17 separator. About two quarts of rich cream were diluted to 10 gallons with water at approximately 100° F., separated, rediluted, and separated until each cream had been washed 14 times or more. Near the end of this procedure, usually at the completion of the twelfth washing, a noticeable oiling off of the butterfat occurred which indi-

cated that the "hull" substances had been largely removed. Further washing appeared to cause a complete oiling off of the butterfat.

Three different butterfats prepared in this way were analyzed for organic phosphorus. For this determination the fats were fused in a fusion mixture composed of two parts sodium carbonate and one part potassium nitrate. After fusion the mixture was dissolved in hot water and hydrochloric acid, and phosphorus was determined by the colorimetric method of Fiske and Subbarow (4). No trace of phosphorus was found in any of the samples, thus indicating that the washing removed all or practically all of the phospholipids adsorbed on the fat globules in the normal milk. In each series the buttermilk and the milk remade by emulsification of washed butterfat in skimmilk were prepared from the same milk that was used as the product of medium phospholipid content. Five such series were prepared and judged.

Regular analyses of each series judged for flavor richness were not made. One series was analyzed, however, to make certain that the expected differences in phospholipid content actually occurred. In this series the cream used was considerably lower in butterfat content than in the other series studied. The phospholipid content was determined as follows: A macro-Roese-Gottlieb extract was prepared according to the method of Bird and Sands (1). The phosphorus content of the fatty extract obtained in this manner was determined by the method described above for the determination of phosphorus in washed butterfat, and the percentage of distearyl

TABLE 2

The quantities of ether-alcohol soluble but acetone insoluble substances in buttermilks from creams of varying richness

MILK	CREAM	FAT CONTENT OF CREAM (BABCOCK)	ETHER-ALCOHOL SOLUBLE, ACETONE INSOLUBLE SUBSTANCES IN BUTTERMILK
		Per cent	*Per cent**
A	1	18	0.2145
	2	43	0.2982
	3	71	0.4874
B	1	17	0.2356
	2	37	0.4578
	3	64	0.5580
C	1	12	0.1652
	2	26	0.1966
	3	66	0.3863
D	1	24	0.2023
	2	39	0.2609
E	1	20	0.2609
	3	66	0.3460

* Average of duplicate determinations.

TABLE 3

The phospholipid contents of a series of three milk products prepared for flavor comparisons

MILK PRODUCT	PHOSPHOLIPID CONTENT	
	Duplicates	Average
	Per cent	*Per cent*
Buttermilk from cream containing 35% butterfat	0.0911 0.0885	0.0898
Normal whole milk	0.0287 0.0291	0.0289
Milk prepared by emulsifying washed butterfat in skimmilk	0.0168 0.0164	0.0166

TABLE 4

Comparison of the richness of flavor of buttermilk and skimmilk standardized to the same fat content (Babcock test) with normal cream and with washed butterfat

ORDER OF FLAVOR RICHNESS	BUTTERMILK*	SKIMMILK STANDARDIZED WITH NORMAL CREAM	SKIMMILK STANDARDIZED WITH WASHED BUTTERFAT
First	20	3	5
Second	5	19	4
Third	3	6	19

* Two of the buttermilks gave a noticeable oxidized flavor.

lecithin was calculated from the quantity of phosphorus found. The results are shown in table 3.

It will be observed that these results are lower throughout than those of Bird and Sands and still lower than those of Holm *et al* (5). This probably was due to the method used rather than to differences in phospholipid contents of the products studied by the different workers. However, the results shown in table 3 indicate the important point concerned in this study, *viz.*, that the samples prepared were of relatively high, medium, and low phospsolipid content, respectively. The phospholipids found in the sample made up from washed butterfat and skimmilk are undoubtedly from the skimmilk inasmuch as the analysis of the washed butterfat as reported above showed no determinable trace of phospholipids.

Each series of milks was judged as soon as it was prepared. The samples were judged by a group of men familiar with dairy products who were not informed of the identity of the samples. They were selected on the basis of their ability to detect differences in flavor richness in standardized milk samples varying in butterfat content from 1 to 4 per cent, with regular differences of 0.5 per cent between samples. Seven were able to

place these samples in their exact order of richness or nearly so and were selected as judges. There were four of these men who judged the experimental milks regularly while one or more of the others usually was available.

The results of judging the five series of milks are shown in table 4. The data show a striking relationship between the method of preparing the samples and their flavor richness. Inasmuch as the samples compared within each trial were standardized to the same butterfat content, on the basis of the Babcock test, it is reasonable to assume that the difference in richness of flavor observed was due to variations in the amounts of "hull substance" present. Because phospholipids are glycerides of a molecular configuration similar to butterfat, and because the order of flavor richness of the samples is directly related to the amount of phospholipids expected to be present due to the methods of preparing the samples, it is indicated that phospholipids contribute to the rich flavor of milk.

In order to make a more direct determination of the effect of lecithin on the flavor of milk, milk lecithin was prepared and dispersed both in skim-milk and in synthetic milk. The milk lecithin was prepared by purification of the alcohol extract of dry buttermilk. Two shotgun cans were partly filled with 98 per cent ethyl alcohol, and 25 pounds of fresh dry buttermilk were stirred into each. The mixtures were allowed to stand for three days, being frequently stirred. Occasional additions of alcohol were made to replace that which had evaporated. At the end of this period the alcohol was removed by means of a hydraulic press operated at a pressure of approximately two tons per square inch. The alcohol solution then was placed in wide, enameled trays and the alcohol evaporated at room temperature with the aid of an electric fan. The residue was taken up in a small volume of ether and, without filtering, precipitated with acetone and pounded together with a glass rod. The acetone-ether mixture was decanted and the precipitate dissolved in ether, precipitated, and treated as before. This procedure was repeated three times to remove all but traces of acetone-soluble compounds. The precipitate then was emulsified in a large excess of water to which a small amount of sodium chloride had been added, after which it was reprecipitated by the addition of a quantity of acetone equal to one-half of the volume of the emulsion. The precipitate then was removed, reemulsified, and treated in the same manner. This was repeated three times. The final precipitate was dried by treating with acetone, as much acetone as possible pressed out, and the whole taken up in ether. The ether solution was allowed to stand in tall cylinders until the white precipitate, sphingomyelin, settled, after which the ether solution was decanted and treated with an excess of acetone. The resulting precipitate again was taken up in ether and the process repeated. The substance then was dissolved in alcohol and any precipitate allowed to settle out, after which the alcohol was evaporated. The residue then was taken up in ether

and precipitated with acetone. Finally it was treated with several changes of acetone and dried in a desiccator, in vacuum over sulphuric acid. The substance thus purified is believed to be a mixture of lecithin and cephalin. However, it possesses all the properties of the substances generally referred to as lecithin.

Synthetic milk was prepared according to the method of Clark (3) except that the casein used was prepared according to the method of Van Slyke and Bosworth (12). To one sample of synthetic milk was added .5 per cent of washed butterfat, prepared as previously described, to a second sample .5 per cent of lecithin was added, and to a third an amount of gum arabic equal to that used to emulsify the washed butterfat in sample one. A similar set was prepared using skimmilk in place of synthetic milk. The judges were unanimous in placing the lecithin samples first, the washed butterfat samples second, and the gum arabic samples last on the basis of rich flavor. They also observed that the samples containing lecithin were distinctly oxidized in flavor and the sample prepared from skimmilk seemed to be identical in flavor to the buttermilk samples in which an oxidized flavor was noted previously.

CONCLUSIONS

That the phospholipids of milk contribute to the richness of flavor in milk products is indicated by the results herein reported.

Buttermilk from sweet cream has a rich flavor probably because of the presence of relatively high percentages of phospholipids left in the buttermilk as the result of their disengagement from the fat globules during churning.

ACKNOWLEDGMENT

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THE VOGT METHOD OF MANUFACTURING FLAKE BUTTERMILK

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The Vogt method of making flake buttermilk was discovered by Gerald Vogt, Assistant Superintendent of the Cohocton plant of the Wetmiller Dairy and Farm Products Company, Inc., Cohocton, New York. It is a simple method of forming flakes or granules of butter in buttermilk that can be employed on either a small or a large scale. It consists of spraying melted butter into cold cultured buttermilk during constant agitation.

DETAILS OF MANUFACTURE

The first step in the manufacture of this product is to make a superior cultured buttermilk. In the manufacture of cultured buttermilk, it is desirable to select good skimmilk. Then, usually, salt should be put into the skimmilk at the rate of two to four ounces per ten gallons of skimmilk. The purpose of adding the salt is to improve the flavor and to make a smoother, or less lumpy buttermilk. At this point the skimmilk should be pasteurized by heating to a temperature of approximately 190° F. for 30 minutes, after which it should be cooled to a holding temperature of about 70° F., when it must be inoculated with a good starter. The incubation or ripening period will require about 12 to 15 hours. An acidity of approximately .75 per cent total acidity expressed as lactic acid, ordinarily is satisfactory.

This curdled skimmilk now should be cooled to approximately a storage temperature of about 40° F. during which time sufficient agitation must be applied to assure a smooth, or lumpless body. It can then be sold as cultured buttermilk or used in making flake buttermilk by the Vogt method.

For the Vogt method, a sufficient amount of good butter should be melted to supply all, or a part, of the milkfat up to 1 per cent. If the buttermilk is to contain 1 per cent milkfat, some of it could well be added in the form of homogenized cream. The milkfat that is to be supplied in the form of flakes should be heated in the sprayer to 120° F., at which time the butter color should be put in. Usually about five to ten times as much color should be used as in the manufacture of butter, for it is essential that the flakes be easily seen. On the other hand, they should not be too highly colored. Mr. Vogt advises that water should be added to the butter, making a mixture of water and milkfat of about one-third water to two-thirds milkfat.

A good sprayer is essential. The one used in this experiment was

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Brown's Auto-spray which is commonly used for spraying fly-killing mixtures, disinfectants, and the like. Other sprayers may do just as well. At this time in the process the water-milkfat mixture should be heated in the sprayer to 180° F. for several minutes, in order to kill the microorganisms in both the mixture and the sprayer. Then the contents of the sprayer should be cooled to about 120° F., when the milkfat may be sprayed directly on to the surface of the cold-cultured buttermilk while it is being constantly agitated. During this stage of the process the sprayer should be shaken often, so that the water and milkfat will be maintained in a reasonably good emulsion. If it is desirable to make a finished product containing one per cent milkfat, or any other definite amount, the loaded sprayer should be weighed and then as the load is reduced in weight, additional weighings should be made.

OBSERVATIONS

The flakes in the buttermilk that have been made by the Vogt method are flat and somewhat ragged in general appearance, and they vary considerably in size. Uniform spraying and agitation are the main factors in controlling the size of the flakes. The particles of butter that are formed in the churning method are more uniform in size, and they are round.

The sanitary feature of the Vogt method is important. The sprayer which is made of metal is readily cleaned and scalded. The wooden churn, on the other hand, is most difficult to keep sanitary, particularly when it is not used daily.

The Vogt-method buttermilk is easily and economically made for the cultured buttermilk from which it is manufactured can be stored, and within five minutes it can be transformed to flake buttermilk. Also, if on account of age or other reason, the original-stock supply goes bad, the milkfat is not lost, for it may be added only when the flake-buttermilk order has been received.

A comparative body and flavor study was made of (a) churned-cream buttermilk, (b) churned-flake buttermilk, and (c) Vogt-method buttermilk. Four sets of five samples each were run in which a sample of each set was examined on five consecutive days.

The body of the churned-cream buttermilk was free from lumps and poured like sweet milk. The other two buttermilks possessed rather heavy bodies with casein particles sufficiently large to appear lumpy. The flavor of the churned-cream buttermilk was preferred by persons accustomed to the "old fashioned" buttermilk in which there is a definite flavor of the butter. The other two buttermilks contained a fatty flavor that approached that of butter. The flavor of all three types of buttermilk was maintained splendidly for five days.

American Dairy Science Association Announcements

ANNUAL MEETING AMERICAN DAIRY SCIENCE ASSOCIATION, UNIVERSITY OF MINNESOTA, UNIVERSITY FARM, ST. PAUL, JUNE 25 TO 27, 1935

GENERAL INFORMATION

The meeting will open officially on Tuesday, June 25th. Registration will begin on Monday, June 24th, and a conference on judging dairy products will be held during the afternoon of that day. There will be no conference on judging dairy cattle this year. Rooms will be available in the College of Agriculture dormitories at 75¢ per person per night. Further details will be published in the succeeding issues of the JOURNAL OF DAIRY SCIENCE.

CALL FOR PAPERS AND ABSTRACTS

This is the first but not final official call for papers for the scientific sessions of the Association. Individual notices will be sent to members or to Department Heads. It is anticipated that scientific programs definitely will be conducted in the subject matter fields of production, manufacturing and extension. Interest is being manifested in possible programs regarding dairy engineering and regarding teaching problems and methods, as announced in the November 1934, and January 1935, issues of this Journal. Tentative plans are under way for a general scientific session to be held jointly with other agricultural science associations that will be in session at the University of Minnesota during the same week. It is possible that this meeting will be called a meeting of Section O of the A. A. A. S. that will also hold its summer meeting at the University of Minnesota during the week of June 24th.

Members are invited to send titles of papers to the program committee. Non-members are permitted to read papers if a member of the American Dairy Science Association is a co-author. All papers must represent original work not previously published.

Titles of papers should be accompanied by or followed by abstracts both of which must be in the hands of the chairman of the program committee by May 1st. Authors are invited to indicate the section before which they desire to present their paper.

The members of the program committee are H. A. Ruehe, J. M. Sherman and L. S. Palmer, chairman. The latter may be addressed at Biochemistry Building, University Farm, St. Paul, Minnesota.

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